Central IDeA States Regional Meeting Poster Abstracts

Abstract # = Poster #

Odd-numbered abstracts are in Session 1 (4pm-5pm)

Even-numbered abstracts are in Session 2 (5pm-6pm)

1. Scientific Focus Area: Allied Health

Title: Effects of Sleep Hygiene Education on Community-Dwelling Older Adults

Authors: Daesha Erskin (Exercise Science, Black Hills State University), Dr. Ashley Pfeiffer, DPT (Exercise Science, Black Hills State University)

Affiliation: INBRE

Text

Background and Objective:
Poor quality of sleep has become more prevalent among the U.S. population with more than a quarter of people suffering from inadequate sleep. Recent research has explored interventions for improving sleep health using educational strategies for younger adults. However, there is limited data on using these same strategies for community-dwelling older adults (CDOA), who have an even greater frequency of sleep problems. In fact, as high as 50% of CDOA suffer from poor sleep quality. Therefore, the goal of our research study is to provide new insights into the ability to change CDOA sleep hygiene behaviors through the use of technology geared specifically toward sleep hygiene education.

Methods:
Thirty randomized participants completed a survey consisting of demographic information, medical history, Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, Sleep Hygiene Index, Perceived Stress Scale, Patient Specific Functional Scale, and Numeric Pain Rating Scale. Objective measurements were taken including blood pressure, O_2 Saturation percentage, heart rate, respiratory rate, height, and weight. All participants watched a 15-minute sleep hygiene educational video. One group received text messages in addition to the video. Throughout the intervention, both groups wore a sleep tracker device in which data was collected regarding sleep regularity, sleep midpoint, sleep duration, and sleep efficiency. After four weeks participants returned to retest all measurements.

Results:
We are currently collecting data for this project and will present preliminary data during poster session.

Discussion and Conclusion:
Conclusions will be based on preliminary data. However, if our study proves to be successful in improving overall sleep, it will provide ideas for simple tools and strategies for older adults and their health practitioners to use to assist in improving sleep which could ultimately improve their health and quality of life.

Acknowledgements

Research reported in this publication was supported by the South Dakota Biomedical Research Infrastructure Network (SD BRIN) through an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103443. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

2. TITLE: Development of fluorescent biosensor to measure Nedd8 dynamics in cells

AUTHORS: Zachary Davis (BHSU), Teagan Hartley (BHSU), Tara Oren (USD), Alyssa Umphlet (BHSU), Jessilyn Monahan (BHSU), Min Kyung Park (BHSU), Yun-Seok Choi (BHSU)

AFFILIATIONS: Black Hills State University (BHSU), University of South Dakota (USD)

SCIENTIFIC FOCUS AREA: Other (Biochemistry)

ABSTRACT:

1. Background and Objective

Nedd8 is a small (9-kDa) protein involved in the regulation of essential pathways, including cell cycle progression and DNA-damage-induced apoptosis, through conjugation to other proteins, primarily Cullin-RING ligases. In living cells, Nedd8 exists in 2 forms: Conjugated Nedd8 (Nedd8 conjugated to another protein) and Free Nedd8 (unconjugated Nedd8). The conjugation of free Nedd8 to its substrate is called neddylation, and the separation of conjugated Nedd8 from its substrate to make free Nedd8 is called deneddylation.

Balancing the rates of neddylation and deneddylation is critical to maintaining homeostasis. Inhibitors of neddylating or deneddylating enzymes have potential for treating diseases caused by imbalance of these processes. Because free Nedd8 is consumed in neddylation and a product of deneddylation, developing a method of quantifying free Nedd8 would enable more effective study of both processes in-vivo and enable large-scale drug screening for neddylation and deneddylation inhibitors.

2. Methods

To this end, we have developed a fluorescent biosensor to quantify free Nedd8 in-vivo and in cell lysates. Our biosensor was rationally designed from a Nedd8-binding protein. It is expressed by *E. coli* cells transformed with a cloned plasmid with the gene encoding our biosensor.

3. Results

We have produced several models of our biosensor with different active site mutations and characterized them using binding assays. Our latest biosensor's K_D for free Nedd8 is 219 ± 46.8 nM. Its K_D for conjugated Nedd8 is 95.1 ± 74.7 μM, 433.5 times greater.

4. Discussion and Conclusions

Our results demonstrate our biosensor preferentially binds to free Nedd8. It can also quantify free Nedd8 at very low concentrations. Future work will focus on applying our biosensor to quantify free Nedd8 in human cell lines, both in-vivo and in cell lysates.

GRANT SUPPORT: This project was supported by South Dakota Biomedical Research Infrastructure Network through an IDeA award from NIH-NIGMS (grant no. P20GM103443)

3. An Initial Assessment of Simple Coumarins as Fluorescent Reporters for HSA Binding

Thomas Gonnella*, Kaitlin Ensign, and Thyra Peterick,

Division of Science and Mathematics

Mayville State University, Mayville, ND

Background and Objective - Human serum albumin (HSA) contains nine fatty acid binding sites, which includes two common drug binding sites and is an essential transport protein for drugs and endogenous compounds to target organs, Examining the binding of drugs to albumin is of high importance because it determines the pharmokinetics, biodistribution and toxicity of a drug. Many HSA binding studies have relied on tryptophan fluorescence quenching to assess drug binding. The HSA protein only contains one tryptophan residue located within subdomain IIA, close to Sudlow's site I. The intrinsic tryptophan fluorescence is sensitive to closely associated ligands, so this fluorescence is measured to probe drug binding at HSA Site I. Unfortunately, this technique does not provide information on many of the other possible sites on HSA and many drugs have primary and secondary binding locations. Our goal is to use fluorescent coumarins as reporters for multiple binding sites on HSA.

Methods - Our group has started to explore using simple coumarins bound to HSA to assess competitive binding of non-fluorescent drugs and fatty acids using time-resolved fluorescence.

Results - Ki values for a few common drugs and fatty acids have been determined based the release of coumarins bound at specific HSA locations.

Discussion and Conclusions - Our initial results support the premise that many drugs bind to HSA beyond a single primary binding site but allosteric binding and conformational changes in HSA appear to be issues in these initial results.

Acknowledgement – The research reported in this publication was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the NIH under grant number P20GM103442.

4. Assessment of Hemp Pollen for Protective Effect in Honey Bee (*Apis mellifera*) Hives

Calvin Belton[1] H Lipp[1], Joanna Gress[1]

[1]Department of Biology, Emporia State University

Background and Objectives

Neonicotinoids have contributed to honey bee decline and are one of the co-morbidities associated with colony collapse disorder (CCD). The most widespread neonicotinoid is Imidacloprid, which is found in over 4,000 registered products, and is associated with decreased honey bee cognition, physiological health, and function of the hindgut. Hemp pollen and its associated phytocannabinoids have been suggested to increase both the activity of antioxidant enzymes in bee hindgut and stress tolerance within the hive. This may negate many of the issues created by neonicotinoids.

Methods

A pilot study was conducted in Summer 2022 to assess hemp pollen's antioxidant properties using 5 treatments: control, commercially available pollen patty only, Imidacloprid + commercially available pollen patty, hemp pollen patty only, and Imidacloprid + hemp pollen patty. For 4 weeks the hives were assessed and forager bees were collected for qPCR analysis of 10 genes in the antioxidant/detoxification pathway in the honeybee gut.

Results

When comparing Imidacloprid + commercially available pollen patty and Imidacloprid + hemp pollen patty, we saw an upregulation in the genes catalase, sod1, and sod2, which are all associated with the primary antioxidant pathway.

Discussion and Conclusions

Preliminary results indicate that hemp pollen may be providing a rescue effect against Imidacloprid exposure through upregulation of the primary antioxidant pathway.

5. Assessing the Effect of Hemp Pollen on Neonicotinoids Impact on Honey Bee Learning and Memory

Meagan Fernandez[1], Hannah Hiszczynskyj[1], Dr. Joanna Gress[1]
[1]Department of Biology, Emporia State University

Background and Objectives

Honey bees, *Apis mellifera,* are increasingly exposed to neonicotinoids, which is one comorbidity of colony collapse disorder. One-fifth of all insecticides globally produced is the neonicotinoid, imidacloprid. Industrial hemp, *Cannabis sativa L.* is currently used in many commercial products. Hemp contains several phytocannabinoids that can affect redox balance by modifying the level and activity of oxidants and antioxidants. Honey bees have been reported to utilize hemp pollen as a protein source, especially in late summer. This may improve survival and pesticide tolerance in honey bees, and a difference might be seen in pollen foraging and foraging rates among hives due to memory loss.

Methods

Hemp pollen was collected from the John C. Horticultural Center in Haysville, KS, through the Kansas State University Industrial Hemp Program. Hives were fed either hemp pollen patties or commercial pollen patties with or without imidacloprid. Hive health was assessed through forager counts and pollen collected in traps.

Results

Overall, hives fed Ultra Bee and imidacloprid collected less pollen and had fewer foragers return to the hive than those fed only sugar water and pollen patties. Hives fed hemp and imidacloprid started out with pollen collection and forager return rates the same as hives fed Ultra Bee and imidacloprid, however in week 3 hives fed hemp and imidacloprid experienced a rescue effect. The P-Value for the significance of pollen collection was 0.010, and the P-Value for the forager return rate was 0.037 both found by using an ANOVA test.

Discussion and Conclusions

The P-Values found for both variables were deemed significant as they were both under 0.05. Since both P-Values from the preliminary data were significant, both pollen collected and forager counts might show that hemp pollen reduces the effects on learning and memory from neonicotinoid stress on honey bees.

Acknowledgments

Special thanks to Dr. Jason Griffin, Director of the John C. Pair Horticultural Center for his help growing and collecting hemp for this study. Thank you to Darren and Alyssa Rebar for allowing us to keep our hive on their farm. Thanks to Kyle Petric for his advice on honeybee management.

This project was supported by an Emporia State University Summer Undergraduate Research Program (ESURP).

This project was supported by an Institutional Development Award (IDeA) from the National Institute of General Medicine Sciences of the National Institute of Health under grant number P20 GM103418.

6. Synthesis of a Hyaluronic Acid-Deferoxamine Conjugate for Local Treatment of Bone Regeneration

Navya Singh, Laird Forrest

Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas

Medically based efforts and alternative treatment strategies to prevent or remediate the corrosive effects of radiotherapy on pathologic fracture healing have failed to produce clear and convincing evidence of success. Establishing an effective pharmacologic option to prevent or treat the development of non-unions in this setting could have immense therapeutic potential. Experimental studies have shown that deferoxamine (DFO), an iron-chelating agent bolsters vascularity and subsequently enhances normal fracture healing when injected locally into a fracture callus in long-bone animal models. However, due to its short half-life and rapid clearance, maintaining DFO at the callus site during peak fracture angiogenesis has remained challenging. In this study, we set out to strategically enhance the therapeutic efficacy of the DFO via covalently attaching the drug molecule hyaluronic acid (HA). HA plays a critical role in cell differentiation, tissue morphogenesis, proliferation, and wound healing. Herein, we first prepared a HA-DFO conjugated deferoxamine (HA-DFO) using a two-step synthesis method. We then examined the biodegradability of the HA-DFO conjugate as compared with the unmodified HA. The drug conjugate was characterized using NMR and spectrophotometry. The conjugates interestingly found to offer the combined therapeutic effects of DFO and HA after a local administration.

7. Comparing the Presence of Antibiotic Resistance Bacteria in Wastewater Systems to Assess the Population Health of Kansas Counties

Audrey Rymer[1], Jonathan Ferguson[1], Garret Rymer[1], Claudia Da Silva Carvalho[1]
[1]Department of Biological Sciences, Fort Hays State University, Hays, KS

The development and spread of antibiotic resistance, as well as the rise of novel and emerging human pathogens, such as COVID-19, are progressively limiting the treatment and the prevention of most bacterial and viral pathogens, threatening essential components of modern medicine. Many multi-resistant microorganisms, such as *Enterobacteriaceae* and *Staphylococcus aureus*, constitute some of the urgent challenges in medicine and can cause both common and severe infections (Hutinel et al., 2019). Studies showed the water in sewer systems can act as an early warning of the outbreak of a disease (Hutinel et al., 2019). Surveillance and tracking of microorganisms in the wastewater are key to the early warning system (EWS). This suggests that the analysis of sewage samples has the potential to serve as a resource-efficient complement to today's clinical surveillance systems of antibiotic-resistant bacteria. For that purpose, the relationship between resistance rates in sewage needs to be established in Kansas counties. Influent and effluent wastewater and municipal water samples were collected. An antibiogram profile was done for each samples. Each sample was analyzed for the presence of Carbapenem-Resistant *Enterobacteriaceae* (CRE) and Methicillin Resistant *Staphylococcus aureus* (MRSA) once a month from treatment plants located in Ellis and Thomas counties. The results from sample analysis from September to December 2022 suggested there is an above average resistance to meropenem, oxacillin, erythromycin, and clindamycin in Ellis county when compared the national averages. Out of the Gram-positive isolates, 41% are suspected to be MRSA isolates indicate an above average in the State of Kansas.

8. Effects of Rurality on Cancer Risk Factors Using Multiscale-Geographical-Weighted-Regression-Model

Jonah K. Amponsah[a], Jeffrey Thompson[a]
[a]University of Kansas Medical Center, Department of Biostatistics and Data Science, 3901 Rainbow Blvd, Kansas City, KS 66160

Abstract

Smoking, drinking, and obesity are modifiable cancer risk factors. To better support people in achieving a healthier lifestyle, a better understanding is needed of the drivers of these factors. Studies show obesity is more prevalent among adults living in nonmetropolitan counties compared to those living in metropolitan counties, suggesting a need to provide more support to people in rural areas. In most studies, the authors assumed the relationship between obesity or other cancer risk factors and the covariates were consistent across the study area. However, the relationship may be more complex than simple rurality and the effect of rurality may differ across different parts of the United States. To overcome this limitation, we explored the multiscale-geographically-weighted-regression-(MSGWR) model to investigate the effects of rurality and covariates such as sex, race, education, and income, on obesity, alcohol usage, and cigarette smoking. We collected 3140 county-level prevalence measures of cancer risk factors from the Institute for Health Metrics. In the MSGWR model, we assume covariates are nonstationary, that the relationship between response and covariates varies from one location to another. Our global results suggest adults living in metropolitan counties have a higher rate of obesity than those living in nonmetropolitan counties, after adjusting for other covariates. Using local models, a lower prevalence of obesity among men living in metropolitan counties exists as compared to those living in non-metropolitan counties. Women in metropolitan counties tended to have a higher prevalence of obesity than women living in nonmetropolitan counties, after adjusting for other covariates. Within metropolitan counties, the local models indicate a higher prevalence of obesity in the Western region of the US than South, after accounting for potential confounding factors. By using MSGWR model, we were able to analyze regional variation in the effect of metropolitan status on obesity, cigarette smoking, and binge drinking.

Acknowledgments

This work is supported by the National Cancer Institute (NCI) Cancer Center Support Grant P30 CA168524, NIH 5P20GM130423 through the Kansas Institute of Precision Medicine, and by a CTSA grant from NCATS awarded to the University of Kansas for Frontiers: The University of Kansas Clinical and Translational Science Institute (# UL1TR002366) The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH or NCATS.

9. Effect of arginine methylation of Serine/Arginine Splicing Factor 1 (SRSF1) on exosomal miRNA enrichment in pancreatic cancer cells

Kritisha Bhandari[1], Wan-Ting Tina Ho[1], Blaine Mooers[2], and Wei-Qun Ding[1]

[1]Department of Pathology, [2]Molecular Biology & Biochemistry, University of Oklahoma Health Sciences Center

Background and objective: Pancreatic ductal adenocarcinoma (PDAC) is one of the most fatal cancer types. Exosomes are small extracellular vesicles that are enriched in certain miRNAs to facilitate PDAC progression. The precise mechanism involved in the selective exosomal miRNA enrichment remains unclear. We recently reported that the onco-protein Serine/Arginine Splicing Factor 1 (SRSF1) regulates selective exosomal miRNA enrichment in PDAC cells. SRSF1 shuttles between cytoplasm and nucleus, dictated by post-translational modifications such as arginine methylation. The objective of this study is to determine whether arginine methylation of SRSF1 contributes to selective exosomal miRNA enrichment in PDAC cells.

Methods: The human PDAC cell lines PANC-1 and MIA PaCa-2 and the pancreatic ductal cell line hTERT-HPNE were used for this study. The methyltransferase inhibitors AMI-5 and EPZ015666 were applied to suppress SRSF1 arginine methylation. Exosomes were isolated using double-filtration/polymer precipitation. qRT-PCR was conducted to analyze miRNA expression. Site-directed mutagenesis was performed to replace methylated arginine (R93, R97, and R109) with lysine and alanine in a flag- or GFP-SRSF1 construct. Cellular localization of SRSF1 was observed using confocal microscopy. The binding of SRSF1 to miRNAs was analyzed using RNA immunoprecipitation assay.

Results: Treatment of PDAC cells with the methyltransferase inhibitors increased SRSF1 cytoplasmic retention and selectively enhanced the level of exosomal miR-1246 and miR-320d, two miRNAs known to be enriched in PDAC exosomes. Interestingly, replacement of three arginine with alanine or lysine in the flag-SRSF1 construct selectively reduced the binding of SRSF1 to miR-1246 and miR-320d in PDAC cells. However, only the alanine replacement increased SRSF1 cytoplasmic accumulation.

Discussions and conclusion: We demonstrate that arginine demethylation of SRSF1 enhances selective exosomal miRNA enrichment likely via altered SRSF1-miRNA binding in PDAC cells. These findings suggest that arginine methylation of SRSF1 is a critical post-translational modification that controls exosomal miRNA signaling initiation in PDAC cells.

Acknowledgments: Funding was provided by pilot grants from the NIH COBRE Oklahoma Structure Biology program (P20GM103640 and P30GM145423).

10. Implantation of intrauterine SHetA2 polymer rod reduces the effect of Estradiol-induced endometrial hyperplasia in rat model system

Debasish Kumar Dey, Danielle Krause, Rajani Rai, Doris Mangiaracina Benbrook, Vishal Chandra

Gynecologic Oncology Section, Obstetrics and Gynecology Department, Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA.

Abstract

Background and Objective: Endometrial cancer (EC) is the most prevalent gynecological malignancy in United States with continuously worsening incidence and mortality. In most cases, atypical endometrial hyperplasia (AEH) has been identified as a risk factor and early precursor for EC. Therefore, reversing EH/AEH to normal endometrium could be a promising approach to prevent EC development. Current treatment options (hysterectomy and hormonal therapy) for EH/AEH has limitations for younger and obese patients. Therefore, identification of non-hormonal, minimally toxic drug could be an advantage. SHetA2 is once such novel, non-toxic drug which has been studied to inhibit cancer cell growth. However, its low aqueous solubility and absorption demonstrated its limitations over oral bioavailability. Therefore, to improve its delivery and absorption, we formulated SHetA2 containing intrauterine device (IUD) for its sustained release over prolonged period to reduce EH/AEH lesions. **Methods:** We formulated four different types of IUDs containing SHetA2 drug and estimated its release in saline over 3 months period. Next, we developed AEH rat model by estradiol (E2) supplementation. After a week of E2 supplementation, we performed oophorectomy in rats and best optimized IUD was implanted in the uterine horn. The change in the bodyweight was recorded every week. After 12 weeks of treatment, necropsy was performed and uterus, liver, spleen, and kidney were collected and weight was recorded. Furthermore, ultrasound imaging was also performed to confirm IUD inside the uterine horn. **Results:** E2 supplementation alone decreased the body, but it significantly increased the uterine weight with respect to its non-E2 group. Upon SHetA2 treatment uterine weight has shown a decreasing trend. **Discussion and Conclusions:** The reduced uterine weight upon SHetA2 treatment suggested the drug has the efficacy to control E2-induced endometrial hyperplasia.

Grant support: The work was supported by “IDeA Network of Biomedical Research Excellence (INBRE) grant P20GM103447”.

11. Veratridine functions as a potential anti-mTORC2-Rictor tumorigenic pathway inhibitor in human colorectal cancer

Morgan Eikanger, Dr. Khosrow Rezvani

Despite advances in treatment regimens, one-third of colorectal cancer (CRC) patients diagnosed with the metastatic form of the disease will die. Understanding the mechanisms underlying the multistep metastatic programs activated in CRC tumors is critical for developing novel therapies that will improve the management of this advanced disease. We have previously shown that veratridine (VTD), a lipid-soluble alkaloid extracted from Liliaceae plants, transcriptionally increases a ubiquitin-like molecule called UBXN2A. UBXN2A functions as a tumor suppressor protein by targeting specific tumorigenic pathways that are overactivated in colon cancer cells. Our recent publication indicates that UBXN2A targets the mTORC2 tumorigenic pathway in CRC by targeting rictor, a key member in the mTORC2 protein complex. We hypothesized that VTD-dependent induction of UBXN2A can inhibit the RICTOR-mTORC2 pathway, which is responsible for tumor growth, migration, and metastasis. VTD increases the level of UBXN2A protein, which decreases the level of rictor protein. This leads to the suppression of mTORC2's downstream pathways. VTD decreases vascular endothelial growth factor (VEGF) levels and the population of positive colon cancer stem cells (CD144+, CD44+, and Lgr5+). An xCELLigence migration experiment in living cells and a biomechanical assay in 3D collagen revealed that VTD successfully reduces cell migration and the cells' ability to generate force and stiffen the matrix. To examine the anti-growth potential of VTD in animal models, we used a mouse model of colorectal cancer (CRC) with progressing tumor masses in the colon and rectum. Vehicle and VTD-treated mice revealed that intraperitoneal injection of VTD (0.1 mg/kg every other day for 30 days) significantly decreases the growth of tumors in C57BL mice. These findings provide an attractive and promising target for the next generation of drugs capable of targeting metastatic CRC.

12. Title: Discovery of a Novel Lead Lactate dehydrogenase-A inhibitor Targeting Pancreatic Cancer

Colter Esparza[1], Uzziah Urquiza[1], Somrita Mondal[2], Hanna Hill[1], Seth Bartlett[2], Lerin Luckett-Chastain[3], Anne Cooper[4], Kevin P. Battaile[5], Scott Lovell[4], Pragya Sharma[1], Michael A. Ihnat[3], and Horrick Sharma*[2]

1. Department of Biological Sciences, Southwestern Oklahoma State University, Weatherford, Oklahoma 73096, United States

2. Department of Pharmaceutical Sciences, College of Pharmacy, Southwestern Oklahoma State University, Weatherford, Oklahoma 73096, United States

3. Department of Pharmaceutical Sciences, College of Pharmacy, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73117, United States

4. Protein Structure and X-ray Crystallography Laboratory, The University of Kansas, Lawrence, Kansas 66047, USA

5. NYX, New York Structural Biology Center, Upton, NY 11973, USA

Background and Objective: Lactate dehydrogenase-A (LDHA) is a glycolytic enzyme and a mediator of the Warburg effect in cancer cells. Although LDHA is strongly associated with tumorgenicity, suppression of LDHA may induce mitochondrial changes and affect OXPHOS. Thus, an effective LDHA inhibitor is needed in a combination therapy to target metabolic plasticity and improve outcomes in metastatic pancreatic cancer patients.

Methods: We used computational, medicinal chemistry, structural biology, and chemical biology approaches to identify a lead LDHA inhibitor.

Results: We identified several scaffolds as novel LDHA inhibitors using virtual screening. Medicinal chemistry led to the discovery of compounds that inhibit LDHA in nM concentrations. We co-crystalized our lead inhibitor with LDHA to confirm the binding mode and target engagement. Compounds inhibit lactate production and show promising cytotoxicity in pancreatic cancer cell lines. The lead compound exhibits in vitro microsomal stability and desirable in vivo pharmacokinetics with low clearance and good oral bioavailability. Metabolic flux assay suggests that our lead compound inhibits basal and compensatory glycolysis. Using a nutrient starvation approach, we determined the effect of glycolytic suppression on metabolic gene expression and observed increased expression of OXPHOS genes. ATP rate assay showed an overall decrease in ATP production rate in MIA PaCa-2 cells when glucose is substituted by galactose. However, we observed an increased contribution of mitoATP relative to glcoATP, suggesting OXPHOS upregulation upon glycolysis inhibition. Interestingly, our compounds exhibit a synergistic effect in a cell viability study with an OXPHOS inhibitor Phenformin.

Discussion and Conclusions: Our lead compound may address the need for a metabolically stable, orally bioavailable LDHA inhibitor with drug-like properties for preclinical studies as a chemical probe. The results support further development of these compounds as a prospective clinical candidate in effective combination therapy against pancreatic cancer.

Acknowledgement: Financial support awarded to Horrick Sharma and Pragya Sharma, by the National Institute of General Medical Sciences of the National Institutes of Health under OK-INBRE award number P20GM103447 is greatly appreciated.

13. The effect of three polyphenols on morphology, proliferation and viability of human lung and pancreatic cancer cell lines

Phillip Harries, Peter Chung, Devapriya Sagaran and Mary Gathoni

Department of Biology, Pittsburg State University

In recent years, there has been much interest in the ability of naturally occurring plant derived polyphenols to inhibit specific type of cancers. In this study, the anticancer properties of the polyphenols curcumin, resveratrol and rutin were evaluated using human lung adenocarcinoma cell line, A549 as well as the human pancreatic cancer cell lines capan-1 and panc-1. The effect of various concentrations of these compounds on cell morphology, proliferation and viability were investigated. A wound healing assay was used to test for inhibition of cell proliferation/migration and the MTT assay was utilized to investigate effects on cell viability. In response to the polyphenol treatments, the A549 lung cancer cells showed the greatest change in cell morphology with little to no observable effect on the pancreatic cancer cells. In both the wound repair and MTT viability assays, the effects varied across the three cell lines with some showing significant response to particular polyphenols. These findings suggest that various cancer cell lines may respond differently to individual polyphenols and highlight the potential benefits of polyphenols in treatment of both primary and metastatic tumors.

14. Knockdown of SOX2 in UROtsa Cells transformed with arsenite decreases the expression of KRT6.

Madison Jones, Kaija Kinnunen, Kaitlyn Berwald, Becker Linder, Danping Guo, Aaron Mehus and Seema Somji. Department of Pathology, University of North Dakota, School of Medicine and Health Sciences, Grand Forks, ND.

Background and Objective. Exposure to inorganic arsenite (As^{3+}) has been linked to the development of urothelial carcinoma (UC). These carcinomas are either muscle invasive (MIUC) or are non-muscle invasive (NMIUC). The MIUC are molecularly sub-typed as being luminal or basal based on gene expression signatures. The basal subtype is generally more aggressive and portrays squamous features with elevated expression of certain keratins. The level of squamous differentiation correlates to patient prognosis and response to chemotherapy and radiation, but the underlying mechanism driving squamous differentiation has not been elucidated. This lab has previously identified that both SRY-box transcription factor 2 (SOX2) and keratin 6A (KRT6A) localize to squamous areas of tumor hetero-transplants derived from As^{3+}- transformed (As-T) urothelial cells. The goal of the study is to determine if the transcription factor, SOX2, may regulate the expression of genes associated with the basal subtype of MIUC with squamous differentiation, such as KRT6A, KRT1, and DSG3.

Methods. Small interfering RNA (siRNA) targeting SOX2 was used on two separate As-T cell lines to knock down or reduce the expression of this transcription factor. Western blotting and RT-qPCR were used to measure protein and gene expression, respectively. Immunofluorescence was used to assess KRT6A protein localization in the As-T cells.

Results. Western blot and qPCR results demonstrated effective knockdown of SOX2 within both As-T cell lines. Furthermore, the knockdown of SOX2 resulted in decreased protein and mRNA expression of KRT6A. The gene expression of DSG3 and KRT1 were unchanged.

Conclusions and Future Directions. These results indicate that SOX2 regulates KRT6A expression in As-T cells. Further in vivo studies are needed to address whether knocking down SOX2 can decrease squamous differentiation in tumors and increase sensitivity to chemotherapy treatment.

15. THE EFFECT DIFFERENCE ON MYOFIBROBLAST DEVELOPMENT BY MARINE SPONGE EXTRACT BETWEEN NOCODAZOLE

Sunghyeok Ko, *Dariana Hashemi, Melville Vaughan*

Department of Biology, University of Central Oklahoma, Edmond, OK

Focused area: Cancer

Introduction: Marine extracts have been researched for utilizing their anti-tumor chemicals and cytotoxic effects, including microtubule disruption. In addition, microtubule disruption was found to promote myofibroblast differentiation while not interrupting transforming growth factor-beta 1 (TGF-β1) signaling. When a myofibroblast is influenced by TGF-β1 it increases alpha smooth muscle (α-sm) actin and functional differentiation, resulting in increasing contractility. The contraction increase was also found in nocodazole treatment, inhibiting microtubules while actin is still active to create tension strength. In this study, we investigated how a marine sponge extract can be used as a microtubule inhibitor and compared nocodazole (a known microtubule inhibitor) effects on TGF-β1 treated fibroblasts. We asked whether this extract affects myofibroblast morphological and functional differentiation.

Methods: Human dermal fibroblasts (HDF00703; UCO IRB-approved study) cultured in log-phase were treated with TGF-β for 2 days, then exposed to marine sponge extract (O2PD1L), Nocodazole, or DMSO vehicle control. Tubulin-β and α-sm actin were assessed using immunofluorescence and western blot assay.

Results: Both O2PD1L and Nocodazole changed the cell shape and caused apoptosis. However, O2PD1L had random fixation on tubulin-β rather than disrupting microtubules like nocodazole. In western blot, O2PD1L and nocodazole had lower concentration ratio compared to vehicle control. Moreover, only O2PD1L had decreased α-sm actin in comparison to control group.

Conclusion: The resultant from the experiment suggests new option on marine sponge extract such as O2PD1L is not only working as anti-mitotic chemicals with microtubule fixation, but also disrupting α-sm actin which has the possibility for actin-targeting drug.

16. Concentrating lambda concatemer DNA utilizing an acrylamide roadblock

Kristy L. Kounovsky-Shafer[1], Samantha Rau[1], and Thi Huynh[1]

1. Department of Chemistry, University of Nebraska – Kearney, Kearney, NE

Background and Objective

Identifying large variations in a cancer genome can be cumbersome. However, using large DNA molecules that span the genomic variations aids in assembling the variation. However, due to the DNA molecule's large size, routine molecular biology techniques can break DNA. Therefore, a method is required to prevent the breakage of DNA during cell lysis and be able to concentrate DNA.

Methods

To help concentrate the DNA, a bis-acrylamide roadblock was cured in the 3D printed device to concentrate DNA at the interface between the roadblock and solution. Lambda concatemer DNA was stained with YOYO-1 and loaded into the 3D printed device. The device was imaged using a Canon camera and a blue light transilluminator.

Results

A dynamic range of voltages was applied to determine how much DNA was concentrated and recovered using fluorescence intensity to determine an optimal voltage to concentrate DNA in the device. The fluorescence of the original solution and the concentrated solution was measured, the recovery was 37% of the original sample. Additionally, the volume decreased by a factor of 3 of the original volume and the large DNA (600 kb) was still intact after the DNA was concentrated.

Discussion and Conclusions

DNA molecules were able to park at the interface between the roadblock and solution, which added the concentration of DNA in the 3D printed devices. The 5× 2× −19 roadblock, with a continuous voltage (15 V), concentrated the most DNA due to the smallest pore size. The concentrated DNA sample can be used for Nanocoding or other sequencing platforms requiring long DNA molecules to assemble genomes or find large structural variations.

17.

Using 3D printed devices to elute and concentrate *S. cerevisiae* DNA

Esmeralda Mendez[1], Thi Huynh[1], and Kristy Kounovsky-Shafer[,1]

1 - Department of Chemistry, University of Nebraska-Kearney, Kearney, NE.

Background and Objective

Identifying large variations in a cancer genome can be cumbersome. However, using large DNA molecules that span the genomic variations aids in assembling the variation. However, due to the DNA molecule's large size, routine molecular biology techniques can break DNA. Therefore, a method is required to prevent the breakage of DNA during cell lysis and be able to concentrate DNA.

Methods

To help concentrate the DNA, a bis-acrylamide roadblock was cured in the 3D printed device to concentrate DNA at the interface between the roadblock and solution. *S. cerevisiae* DNA was stained with YOYO-1 and loaded into the 3D printed device. The device was imaged using a Canon camera and a blue light transilluminator.

Results

S. cerevisiae was tested to determine how much DNA could be eluted and concentrated in the 3D-printed device. The DNA was stained with YOYO-1 dye to track the progression of DNA through the device. The 3D-printed device was affixed to a glass slide, and an acrylamide "roadblock" was used to slow down the progression of DNA. The DNA insert was added to the device, and DNA was eluted into the solution and concentrated in front of the acrylamide roadblock. The DNA sample concentration was measured to determine how much DNA was eluted.

Discussion and Conclusions

The fluorescence images were analyzed with ImageJ to determine how much DNA was eluted, concentrated, and recovered. The DNA inserts were run on a PFGE to determine how much DNA remained in the insert and which size of molecules eluted from the insert.

This research was made possible by grants from the National Institute for General Medical Science (NIGMS) (GM103427), a component of the National Institutes of Health (NIH).

18. The biotoxin BMAA promotes mesenchymal transition in neuroblastoma cells

Ellie Moore[1], Hayes Hansen[1], Jordan Brooks[1], Matthew Pawlus[1]

[1]Department of Natural Sciences, Black Hills State University, Spearfish, SD

Background and Objective: Mesenchymal-like cancer cells are an indicator of malignant tumors as they exhibit several tumorigenic properties including downregulation of differentiation markers, and increased colony-forming potential, motility, and chemoresistance. We have previously demonstrated that the cyanobacterial biotoxin beta-methylamino-L-alanine (BMAA) is capable of influencing neural cell differentiation state through mechanisms involving the Wnt signaling pathway suggesting the possibility that BMAA may play a role in influencing other differentiation processes involving Wnt including mesenchymal transition.

Methods: In this study we present evidence characterizing the effects of BMAA on mesenchymal transition in a human neuroblastoma cell line and provide support for the hypothesis that the biotoxin can promote this process in these cells by altering differentiation state, inducing changes in gene expression, and changing cellular function in manners consistent with cellular mesenchymal transition.

Results: Results of this study indicate that BMAA exposure may promote carcinogenesis through its effects on cell differentiation state in certain contexts.

Discussion/Conclusion: These results suggest that exposure to the biotoxin BMAA may be an influencing factor in chemotherapy resistance and cancer progression in neuroblastoma.

Funding: Research reported in this publication was supported by the South Dakota Biomedical Research Infrastructure Network (SD BRIN) through an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103443. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Cell culturing and functional assays were performed by WestCore at Black Hills State University.

19. In vivo quantification of metabolic and tissue architectural changes associated with UVA-induced skin cancer in SKH1 mice

MG Nichols[1,2], N Abdessalam, MB Barai, TA Gafurova, T Grabher, HM Hubbs, J Li, AC Nguyen, GL Porter, SJ Rogers, DA Remitar, HM Schloman, MK Schultz, DR Snyder, FM Sun, TQ Tran, GJ Varghese , DH Woods, LA Hansen[2]

Creighton University, Department of Physics[1] and Biomedical Sciences[2], Omaha NE 68178

Abstract

Background and Objective: Several hallmarks of cancer reflect both the distinct shift in cellular metabolism and remodeling of the extracellular matrix to accommodate increased growth and proliferation. We developed an imaging approach that allows simultaneous assessment of NAD(P)H to discern shifts in metabolism and collagen by two-photon excited fluorescence lifetime imaging (FLIM). We conducted a longitudinal in vivo study to begin to evaluate the pre-clinical feasibility and potential diagnostic advantage of this approach to a non-invasive optical biopsy.

Methods: A cohort of 30 age-matched SKH1 mice were randomly assigned to UV- and sham-treatment groups. Mice in the UV-treatment group received predominately UVA light (85%/14%/1% UVA/B/C) 5 days-a-week. Phasor-FLIM (NAD(P)H and collagen) and collagen SHG images were obtained throughout the epidermis. The average protein-bound:free NAD(P)H ratio was computed for each image. Additionally, descanned second harmonic generation (SHG) images were obtained using confocal apertures of various diameter and analyzed to estimate the ratio of forward:backward scattered SHG emission. Over 5000 images were acquired throughout the 60-week study and results were compared against histological and immunofluorescence analysis.

Results: In vivo phasor-FLIM imaging revealed an unexpected fluorescent signal associated with collagen. The additional component results in a deviation of the phasor from the free-bound NADH chord of the universal circle. A sharp decline in the NAD(P)H bound fraction and rise in collagen was observed when aggressive mice fought, but both measures returned to baseline after the incident. Few papillomas developed and there were no significant differences between the chronic-UVA and sham treatment groups.

Discussion and Conclusions: An imaging workflow was developed to follow individual mice for over a year. The NAD(P)H bound fraction decreased systematically with imaging depth. Individual mice with visible papillomas tended to have lower bound-fractions and higher collagen signals but the effect was small and no statistically-significant differences were found between UVA- and sham-treatment groups.

20. Single Molecule Studies of PCNA and CAF-1 Using TIRF Microscopy

Olivia Nicholson[1], Dr. Lynne Dieckman[1], olivianicholson@creighton.edu
1 - Department of Biochemistry, Creighton University, Omaha, NE.

Background: Shortly after replication, newly synthesized DNA is packaged and stored in structures called nucleosomes, which are the fundamental units of chromatin. Nucleosomes are composed of double-stranded DNA wrapped around eight histone proteins. This packaging process, called replication coupled nucleosome assembly, is crucial for protecting DNA and maintaining genomic stability. The level of compaction between these nucleosomes also determines which genes are expressed and which are silenced. Genomic regions of loosely packed nucleosomes are typically expressed, while regions of tightly packed nucleosomes are usually silenced. Two proteins that play major roles in replication coupled nucleosome assembly are chromatin assembly factor-1 (CAF-1), a heterotrimeric histone chaperone protein that deposits histone proteins onto DNA for nucleosome assembly, and proliferating cell nuclear antigen (PCNA), which binds and recruits CAF-1 to the replication fork. The interaction between PCNA and CAF-1 is essential for proper DNA packaging and gene silencing; however, the specific mechanism of binding between these two proteins is not known.

Methods: We are using single molecule total internal reflection fluorescence (TIRF) microscopy to determine the binding kinetics and affinity of the interaction between CAF-1 and PCNA.

Results & Discussion: We have built a TIRF microscope system, verified the attachment of proteins to the surface of a microscope slide, and captured the interaction between individual PCNA and CAF-1 molecules. We are currently optimizing these assays to confirm the detection of fluorescently labeled PCNA binding to immobilized CAF-1. Future studies will use these TIRF assays to determine the binding kinetics of the CAF-1-PCNA interaction using varying concentrations of wildtype and mutant forms of these proteins to map their specific site(s) of interaction.

Grant Support:
IDeA Networks of Biomedical Research Excellence (INBRE) Scholar Program
Nebraska-INBRE Developmental Research Project Program (DRPP) Award

21. Analysis of Target Genes in SV-40 Transformed Murine Fibroblast Cell Lines

Carlie Payne and Peter A. Chung
Department of Biology, Pittsburg State University

While many advances have been made in the field of cancer research, more information is needed to fully understand the mechanisms behind metastatic activity. Our research involves mouse fibroblast cells transformed with the SV40 virus. Transformation of cells with SV40 has been proven to cause cancer. After the cells were transformed, fifteen fibroblast sarcoma cell lines emerged. Our focus is on two cell lines, F5b and F5m. F5m is resistant to macrophage-mediated-cytotoxicity while F5b is sensitive to macrophage-mediated-killing. By understanding what mechanisms contribute to macrophage sensitivity in the F5b cell line versus the F5m cell line, we hope to identify therapeutic and treatment options for cancer metastasis. Unpublished microarray data provided by Kansas State University has shown alterations in the expression of many target genes; genes that were either upregulated or downregulated in the macrophage-sensitive cell line F5b compared to the F5m cell line. Most recently Western blot analysis has been mostly inconclusive for most gene targets we have worked with, resulting in ongoing verification. This study aims to clarify some of these discrepancies in our gene target analysis.

22. Title: Exploration of the Thermodynamics of the Surface Water Molecules for the Discovery of Novel LDHA Inhibitors

Authors: Landon Santa-Pinter and Horrick Sharma*, Department of Pharmaceutical Sciences, College of Pharmacy, Southwestern Oklahoma State University, OK 73096

Background and Objective: Lactate dehydrogenase-A (LDH-A) is upregulated in most tumors and executes the last step of aerobic glycolysis by catalyzing the conversion of pyruvate to lactic acid. Although some LDHA inhibitors are known in the literature, only a few have been shown to possess cellular activity. Further, the development of these molecules has been hampered either because they contain some reactive functional groups, lack drug-like properties, or the structure was metabolically susceptible, leading to poor pharmacokinetic profile. Thus, greater exploration of the significantly sizeable chemical space is needed to discover molecules with drug-like properties to target the Warburg effect for cancer therapy.

Methods: In our study, we performed a virtual screening of the ZINC database containing 15 million virtual compounds and identified several new chemotypes as LDHA inhibitors. Here we present one of our computational studies involving WaterMap that calculates the thermodynamic properties, including the entropy, enthalpy, and free energy of crystallographic water molecules in the LDHA binding site.

Results: We determined key hydration sites on the protein surface that are energetically unfavorable. We performed a retrospective WaterMap study and identified amino acids that could be targeted for ligand binding. Molecular dynamics simulations revealed the binding mode of the inhibitors identified in our lab, including interactions with some of these key amino acids.

Discussion and Conclusions: This study is currently being used to design new analogs to develop more potent LDHA inhibitors with amenable drug-like properties.

Acknowledgement: Financial support awarded to Horrick Sharma, by the National Institute of General Medical Sciences of the National Institutes of Health under OK-INBRE award number P20GM103447 is greatly appreciated.

23. EFFECT OF CURCUMIN ON TRANSCRIPTIONAL TARGETS IN THE NFκB AND TGFβ PATHWAY

Emmanuella Tchona, Joshua Krutz, Isioma Akwanamnye, Lelisse Umetta and Ann Marie Buchmann, emmanuella.tchona@eagles.csc.edu, Department of Physical and Life Sciences, Chadron State College, Chadron, Nebraska

Scientific Focus Areas: Cancer

Background and Objective

Triple negative breast cancer (TNBC) accounts for 10-15% of all breast cancer cells. Known as triple negative because of the lack of expression of estrogen, progesterone and the human epidermal growth factor (HER2) receptors, it is diagnosed late, has a faster growth rate, limited treatment options and worse outcomes. Novel therapeutic approaches in our lab aim to target specific proteins in pathways like NFkB and TGFb that play a role in the cell cycle and triple negative breast cancer by affecting the growth rate observed in TNBC cells. Previous experiments with curcumin, the active ingredient in turmeric has demonstrated its anti-inflammatory and tumor suppressing effects. The work done in our labs shows that curcumin inhibits breast cancer growth in cell lines MDA-MB231, causing them to go into apoptosis. Our hypothesis is that curcumin may inhibit the NFkB and TGFb pathways. To test this hypothesis, we are examining the expression of genes regulated by the NFkB and TGFb pathways. Using q-PCR we are quantifying changes in the expression of the following transcriptional targets of the NFkB and TGFb pathways: Cyclin D-family members, Bcl-family members, c-myc, and the Follistatin Regulated Gene (FLRG).

Materials and Method

MDA-MB 231 TNBC cells were treated with different concentrations of curcumin (0μM ,2.5μM, 5μM, 10μM,20μM, 40μM) harvested within 24 hours. RNA was extracted and converted to cDNA. RT-qPCR is used to quantify gene expression with GAPDH serving as a housekeeping gene control. The 2-ΔΔCT method was used to analyze the relative changes in gene expression from RT-qPCR.

Results

Results from past experiments show that the expression levels of cyclin D1 and c-myc at concentrations (4μM,8μM,12μM & 16μM) were decreased compared with those of the control group. The results from our analysis with the new concentrations and the other genes will be presented at the meeting.

Discussion

Curcumin can decrease the expression of gene induced by the NF-κB pathway in TNBC. The results from our analysis with the new concentrations and the other genes will be presented at the meeting.

Grant Support

NE-INBRE NIH grant number 2P20GM10342714A1

24. Behavioral Research Core Facility: Infrastructure and Advanced Equipment for Behavioral Phenotyping in Rodents

Van A. Doze, Chris W.D. Jurgens, Don A. Sens, and Ellen M. Olson, Department of Pathology, University of North Dakota School of Medicine & Health Sciences, Grand Forks, ND 58202

The Behavioral Research Core Facility (BRCF) at the University of North Dakota School of Medicine & Health Sciences is a state-of-the art facility where investigators can conduct behavioral phenotyping for both mice and rats. The BRCF is located in the basement of Columbia Hall. The facility consists of multiple rooms, which are directly accessible from the institution's animal facility. These rooms include four testing rooms which allow simultaneous usage of the facility by multiple research groups. Three rooms are designated for mice and one room for rats. The rat behavior suite is located within the Center for Biomedical Research. The BRCF has 25 different pieces of specialized equipment and offers over 30 different behavioral tests. Available tests include models of attention, learning and memory, anxiety, depression, locomotor activity and coordination, food intake, and metabolic measures. There is also a tissue preparation room and a surgical suite with stereotaxic instruments and an adjacent vivarium room for housing. The BRCF is a full-service core managed by Senior Research Specialist, Ellen Olson.

The BRCF aims to **promote research productivity** and **improve STEM training** in behavioral science by providing for the following needs: Well-managed and maintained equipment; methodological and technical expertise; training in behavioral testing and analysis; interface for interaction of researchers to facilitate collaborations.

The facility is supported in part by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103442.

25. THE OK-INBRE BIOINFORMATICS CORE FACILITY

Dyer, David W., MaJoi Trammell, Michael Day, and Darrin Akins

Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma

Background and Objective: The Oklahoma IDeA Network of Biomedical Research Excellence (OK-INBRE) Bioinformatics Core Facility provides state-of-the-art bioinformatics research and educational support to the state of Oklahoma and the Central States INBRE region.

Methods: *Research support* has focused on: 1) Gene expression analysis using bulk RNA-seq and single-cell RNA-seq in bacterial and eukaryotic systems. 2) Microbiome analysis using 16S rRNA sequencing and taxonomic determinations, focusing on murine and human microbiome samples. 3) Genome annotation and analysis of bacterial and viral pathogens. 4) Protein structure-function predictions, including bacterial DNA binding proteins and bacterial membrane proteins. *Educational support* included lecture and computer laboratory materials focused on: 1) analysis of DNA barcoding data, 2) microbial ecology using 16S rRNA sequence, 3) instructional materials for protein structure and phylogenetic analyses, and 4) microbial genome sequence annotation and analysis.

Results: During the present funding period, the core 1) maintained on-line Ingenuity Pathway Analysis tools and provided tutorials for over 150 faculty, postdocs and graduate students in the state of Oklahoma, 2) provided bioinformatics support for at least 20 research projects on the OUHSC campus, and 3) developed and participated in two bioinformatics courses offered to graduate students on the OUHSC campus (BIOC6321 Survey of Data Science and MI6401 Introduction to Bioinformatics. These courses have provided instructional material to over 20 graduate students on the OUHSC campus.

Discussion and Conclusions: The OK-INBRE Bioinformatics Core has stimulated both basic research and bioinformatics education in the State of Oklahoma, and contributed to the success of the statewide INBRE initiative.

This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447.

26. Title: UNMC Genomics Core Technologies / Opportunities for NE-INBRE Investigators

Authors: James Eudy PhD[1,2], Jennifer Bushing[1], Alok Dhar PhD[1], Xiaoyan Feng[1], Greg Kubik[1], Ronald Redder[1], and Eric Tom PhD[1]

Affiliations: 1) UNMC Genomics Core Facility / University of Nebraska Medical Center, Omaha, NE and 2) Department of Genetics Cell Biology and Anatomy / University of Nebraska Medical Center

Text: 1) Background and Objective: The UNMC Genomics Core Facility is a comprehensively equipped facility that provides genomic services to the members of the INBRE program as well as the greater academic research community in the State of Nebraska. 2) Methods: The next generation sequencing instrumentation includes three Illumina sequencers of different capacities: a high capacity NovaSeq6000, a NextSeq550, and a MiSeq II DNA sequencer. In addition, the core operates the 10x Genomics single cell platform the 10x "X". For targeted gene expression experiments, an NCounter Max instrument from Nanostring is utilized. Collectively these systems allow for a wide variety of genome-wide and targeted gene expression (mRNA, total RNA, and miRNA) and DNA sequencing related experiments. 3) Results: The presence of these cores currently supports the research of over 150 independent laboratories annually which are engaged in both biomedical and basic science research. 4) Discussion and Conclusions: The core has observed tremendous growth throughout the last 21 years due to the support of the IDeA program grants, in particular the INBRE. This funding has been critical to the development and the steady and sustained support of the functional genomics infrastructure in Nebraska.

Acknowledgements: The University of Nebraska DNA Sequencing Core receives partial support from the National Institute for General Medical Science (NIGMS) INBRE - 5P20GM103427-21 grant as well as The Fred & Pamela Buffett Cancer Center Support Grant - 5P30CA036727-36. This publication's contents are the sole responsibility of the authors and do not necessarily represent the official views of the NIH or NIGMS.

27. Equipment and Services of the Kansas University Nanofabrication Facility

Ryan Grigsby[1] and Susan M. Lunte[1,2,3,4]

[1]The Center for Molecular Analysis of Disease Pathways, [2]The Ralph N. Adams Institute for Bioanalytical Chemistry, [3]Department of Pharmaceutical Chemistry, [4]Department of Chemistry, University of Kansas

The Kansas University Nanofabrication Facility (KUNF) is a Core Lab supported by the KU Office of Research and the Center for Molecular Analysis of Disease Pathways COBRE. The KUNF primarily caters to researchers who are manufacturing micro- and nanofluidic devices for biomedical research, but has the equipment and resources to accommodate broad research applications with micro- and nanofabrication needs. The core lab consists of about 1,300 ft^2 of ISO class 5, 1,700 ft^2 of ISO class 6 and 1,250 ft^2 of ISO class 7 cleanroom space, housing tools and materials for techniques including photolithography, nano-imprint lithography, plasma (dry) etching (ICP-RIE), wet etching, metal and dielectric material thin film deposition, scanning electron microscopy (VP-SEM), contact angle goniometry, ellipsometry, profilometry, wafer dicing, laser ablation and engraving, 3D printing, hot embossing, and COMSOL software for device modeling. In addition, the facility has numerous microscopes for general inspection, ovens and furnaces, ultrapure water, dedicated process fume hoods and filtered lighting for photolithography.

This facility is under the direction of Dr. Susan Lunte. Services and usage of the facility are available to researchers from all Kansas universities. Training is provided to new investigators and graduate students in the use of micro- and nanofabrication procedures and equipment. In addition, researchers from both non-Kansas academic and private industry institutions may contract with the facility for consultation and services. Hourly and per-use rates apply for facility access, equipment usage, and staff labor. Consultation is free.

28. Facilitating Research On Substance Use: The Longitudinal Networks Core (LNC) Service Center At The University Of Nebraska-Lincoln

Patrick Habecker

Research Assistant Professor

University of Nebraska-Lincoln

Co-Director, Longitudinal Networks Core

Rural Drug Addiction Research Center

Kimberly A. Tyler

Willa Cather Professor of Sociology

University of Nebraska-Lincoln

Co-Director, Longitudinal Networks Core

Rural Drug Addiction Research Center

Abstract

Background Research on substance use in the United States has often focused on urban areas, or more recently the Appalachian regions. The Rural Drug Addiction Research Center (RDAR) works to further interdisciplinary research on substance use in the Great Plains of the US, focusing specifically on southeast Nebraska. Within RDAR, the Longitudinal Networks Core (LNC) provides a set of resources to facilitate research with people who use drugs (PWUD), develop research software, and expertise in hard-to-reach populations.

Methods The LNC reduces barriers to substance use research and expanding engagement in substance use and related health disparities research in three ways. 1) The LNC recruits and maintains a longitudinal cohort of people who use drugs. Participants complete surveys every 6-9 months on a range of substance use, health, and social network topics. 2) Cohort members may also volunteer to join a participant pool of PWUD from which other researchers working with the RDAR center may recruit interested participants. 3) The LNC has cutting-edge software to collect and analyze data that provide unique insights into dynamic patterns of substance use. The cornerstone of these is ODIN, a unique mobile app, designed to map social network interactions and facilitate responsive ecological momentary assessment.

Results The LNC has recruited over 700 PWUD who have completed the first survey, and 97% of those have joined the participant pool for other studies. We have worked with six studies that have recruited from the participant pool on a wide range of subjects including: stress, fMRI measurement, audiology testing, and medication-assisted treatment. To date, the ODIN mobile software has been used in seven independent deployments, several involving PWUD.

Conclusion The LNC has a successful record of assisting researchers with PWUD data, recruiting PWUD, and mobile data collection approaches. LNC assistance has directly contributed to two R01 awards.

Acknowledgments*: This work was supported by the National Institute of General Medical Sciences of the National Institutes of Health [P20GM130461] and the Rural Drug Addiction Research Center at the University of Nebraska-Lincoln. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the University of Nebraska.*

29. Next Generation Sequencing at KU Genome Sequencing Core

Hackett, Jennifer L. [1,2,3], Kristen M. Cloud-Richardson[1,2,3], Erik A. Lundquist[1,2,3], Susan M. Lunte[1,4,5]

[1]Center for Molecular Analysis of Disease Pathways, [2]Genome Sequencing Core, [3]Department of Molecular Biosciences, [4]Department of Chemistry, [5]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence KS, USA

The Genome Sequencing Core (GSC) is one of three research service core labs in the NIH COBRE Center for Molecular Analysis of Disease Pathways (CMADP) at the University of Kansas (KU). The major mission of the GSC is to provide researchers with next-generation sequencing (NGS) technologies. NGS, carried out in a massively parallel fashion, has been revolutionizing bio-medical research and used in a growing list of applications. Projects supported by the GSC include de novo genome assembly, genome re-sequencing for identification of mutations and polymorphisms, transcriptome analysis (RNA-seq), and epigenomic and gene regulation studies such as ChIP-seq, Methyl-seq, and small RNA analysis. The GSC enhances the genomics infrastructure already at KU by providing a range of Illumina sequencing platforms including the NextSeq 2000 and NextSeq 550 (mid-sized genome re-sequencing or transcriptome projects) and the MiSeq (metagenomic or targeted amplicon sequencing projects) to researchers at KU-Lawrence and across the region. To capture the full power of NGS, we provide a range of project support, including project consultation, sample quality check, sequencing library construction, Illumina sequencing, and FASTQ generation and demultiplexing. For latest pricing, current sequencing queue, or other information, visit the Genome Sequencing Core's website: https://gsc.ku.edu/.

30. Advanced Microscopy Core Facility at the University of Nebraska Medical Center

Heather Jensen-Smith PhD[1], Janice Taylor[2], James Talaska[2]
1) AMCF Director, Eppley Institute, FPBCC, University of Nebraska Medical Center, Omaha, NE 68198
2) Instrument Specialist, University of Nebraska Medical Center, Omaha, NE 68198

Background

The Advanced Microscopy Core Facility (AMCF) is a shared research resource at the University of Nebraska Medical Center (UNMC) offering a full spectrum of biomedical imaging modalities capable of capturing complex biological phenomena occurring at single molecule (nanometer, super resolution), sub-cellular and cellular resolution (micron, confocal and whole slide scanning), and tissue/small organ levels (millimeter, light sheet).

Methods/Instrumentation

State-of-the-art imaging technologies in the UNMC AMCF include a Zeiss ELYRA PS.1 inverted microscope designed for super-resolution (SR) structured illumination microscopy (SIM) and single-molecule localization microscopy (SMLM). The Zeiss 800 confocal laser scanning microscope (CLSM) with Airyscan detector dramatically increases conventional confocal image resolution to ~180 nm for high-resolution imaging exceeding the imaging capacities of the available Zeiss 710 CLSM offering multi-channel and spectral, co-localization, live cell, 3D, and time series imaging. The Zeiss Cell Discoverer7 widefield imaging system provides automated, time-lapse imaging of live samples in dishes and multi-well plates. A whole slide scanning system (Zeiss Axioscan 7) captures transmitted and fluorescent light images of whole sections. The Miltenyi Biotec Ultramicroscope II provides imaging of intact tissues, organs, and small organisms using light sheet microscopy. Additional image analysis workstation (HALO, IMARIS) allow researchers to optimally reconstruct, analyze, and quantify biological changes.

Conclusions

Shared instrumentation and training opportunities in the UNMC AMCF provide Nebraska researchers unique and rigorous opportunities to learn the fundamental strengths and limitations of individual imaging methodologies, how to utilize appropriate instrumentation to rigorously test hypotheses, and how to collect and analyze individual and/or complementary imaging dataset acquired across the diverse array of available technologies.

Funding

University of Nebraska Medical Center - UNMC Advanced Microscopy Core Facility, RRID:SCR_022467, P20 GM103427, P30 GM106397, P30 CA036727, S10 RR02730, S10 OD030486, Nebraska Research Initiative.

31. Multiphoton Intravital and Tissue Imaging Core at the University of Nebraska Medical Center

Heather Jensen-Smith PhD
MITI Director, Eppley Institute, FPBCC, University of Nebraska Medical Center, Omaha, NE 68198

Background

The Multiphoton Intravital and Tissue Imaging (MITI) shared research resource at the University of Nebraska Medical Center (UNMC) with experienced research professionals and state-of-the-art intravital imaging equipment to acquire advanced descriptive and quantitative data from live animals and/or fixed samples requiring deep tissue illumination.

Methods/Instrumentation

The Olympus FVMPE-RS Multiphoton Microscope is a multichannel, multiphoton excitation system specifically tailored for intravital and deep tissue imaging. It delivers high speed excitation (up to millisecond imaging) for rapid in vivo imaging of physiological responses and near infrared (NIR) excitation for deep tissue imaging. The dual NIR, multiphoton system and detectors allow simultaneous image capture of up-to four different fluorophores (blue (410-455 nm), green (495-540 nm), short reds (575-645 nm) and long reds (660-750 nm) with detection capacities extending to 850nm. Various objectives support intravital and multi-immersion (optically cleared) imaging. A specialized neurotar mobile home cage allows researchers to perform real-time imaging of neuronal activity concurrently with activity tracking.

Conclusions

Shared instrumentation and training opportunities in the UNMC MITI provide Nebraska researchers unique and rigorous investigate complex biological phenomena occurring in live and fixed samples. As the only shared research resource in Nebraska with a dual-line NIR laser system, the MITI offers researchers unparalleled opportunities to image various types of biological samples and events.

Funding

University of Nebraska Medical Center - UNMC Multiphoton Intravital & Tissue Imaging (MITI) Core, RRID:SCR_022478, P30 GM127200, P20 GM130447, P30 CA036727, Nebraska Research Initiative.

32. The Computational Chemical Biology Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory

David K. Johnson[1,2] (dkjohnson@ku.edu)

[1]Computational Chemical Biology Core, University of Kansas, Lawrence, KS, USA; [2]Molecular Graphics and Modeling Lab, University of Kansas, Lawrence, KS, USA

Part of the Chemical Biology of Infectious Disease COBRE at the University of Kansas, the Computational Chemical Biology Core (CCB) works in collaboration with the Molecular Graphics and Modeling (MGM) Laboratory to provide the computational resources and expertise to enhance the productivity of researchers studying infectious diseases, in addition to other projects. The CCB has the tools and expertise to perform virtual screening, small molecule docking, chemoinformatics analysis of high-throughput screening hits, binding site prediction, protein/peptide/antibody modeling and docking (including Alphafold modeling), protein design, and molecular dynamics simulations.

Recent highlights include the identification inhibitors of ACMS decarboxylase and DNAJA1 via virtual screening, using modeling to identify the functional activity of *Legionella pneumphila* effector protein SidI, using modelling to assess the structural impact of clinically relevant point mutations of TRIM32, modeling the interaction between the Type III secretion system basal body and sorting platform proteins SctK and SctD from *Pseudomonas aeruginosa*, and the optimization of an inhibitor of PTPRD.

With the software and expertise to perform virtual screening, protein-small molecule docking, protein/peptide modeling/docking, and cheminformatic analysis, the CCB is a valuable resource to enhance the productivity of researchers studying infectious diseases, in addition to other projects.

The CBID COBRE is funded by the NIH NIGMS grant 1P20GM113117.

33. INBRE Microscopy Core Facility at the University of North Dakota: Advanced Equipment for Light Microscopy

Chris WD Jurgens, Van A Doze, Don A Sens, Department of Pathology, University of North Dakota School of Medicine & Health Sciences, Grand Forks, ND 58202

The North Dakota INBRE Microscopy Core Facility (IMCF) at the University of North Dakota provides advanced light imaging technologies to support cutting-edge research in the sciences. The IMCF serves researchers from diverse institutions and fields, and different experience levels. The core is located on the 4th floor of the School of Medicine & Health Sciences Building. The facility is open to all members of the research community, with priority given to IDeA-funded investigators and projects.

The IMCF provides tools for routine fluorescence and confocal microscopy (including live cell imaging), laser microdissection, stereology and has dedicated high performance workstations to support available image analysis software. Near-IR confocal microscopy will soon be offered. Adjacent laboratory space allows outside investigators to prepare cells for viewing using both available bench space and the cell culture and related facilities of the Department of Pathology. The IMCF also provides training and consultation services to help researchers of all levels to collect, analyze, and interpret their images.

The IMCF aims to **promote research productivity** and **improve STEM training** in imaging science by providing: well-managed and maintained equipment; methodological and technical expertise; training in image acquisition and analysis; and an interface for interaction of researchers to facilitate collaborations.

The facility is supported in part by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health (P20GM103442), the Department of Pathology, the UND School of Medicine and Health Sciences and the North Dakota Cancer Collaborative on Translational Activity (DaCCoTA).

34. Capabilities of the IDeA National Resource for Quantitative Proteomics

Michael Kinter[1], Ricky Edmondson[2], Sam Mackintosh[2], Aaron Storey[2], Dennis Province[2], Stephanie Byrum[2], and Alan J. Tackett[2]
[1]Aging and Metabolism Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK
[2]Department Biochemistry & Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR

Background and objective

Proteomics approaches can include detection and quantification of the proteins present and the specific posttranslational modifications (PTMs) at a certain cellular state, as well as the proteins coming together in specific organelles or macromolecular protein complexes. Quantitative proteomics enhances these experiments by determining the amount of protein or protein PTM in those samples.

Methods

Our Resource provides a full range of services for protein characterization by mass spectrometry, including protein identification, mapping of post-translational modifications, global proteomics, and quantitative comparison of proteins in biological samples - all using state-of-the-art equipment. Specific service lines include 1) Discovery proteomics for the large-scale identification and quantification of proteins or protein posttranslational modifications. 2) Targeted proteomics to quantify levels of a small set of target proteins in a larger number of samples. 3) Bioinformatics for the analysis and interpretation of complex datasets to answer the biological question.

Our services are comprehensive, including sample preparation starting from cultured cells, frozen or FFPE tissue samples, serum or plasma, IP and other affinity purifications. Comprehensive data analysis is also included with all services.

Working with the resource

We are committed to working with each investigator to adapt our approach to fit specific research goals and to providing results in customized publication-quality form. All experiments begin with a consultation with Resource staff to help understand the experiment and recommend the best course of action. The price of the experiment depends on number of samples and desired analysis, but are typically in the range of $2000 for a 20sample experiment. (We have an active voucher program to help fund pilot experiments.) From there, samples are submitted to the Resource for processing, analysis, and bioinformatics. Results are reported in an interactive series of figures including heat maps and volcano plots. Typical turnaround times are in the range of 4-6 weeks.

Acknowledgement

The IDeA National Resource for Quantitative Proteomics is supported by a grant from the NIH (R24 GM137786).

35. Title: Intentionality in Mobile Research: Defining the Program Theory for the Boys Town Research Vehicle (BTRV)
Authors: Randi Knox, MS [presenting author]; Angela Collins, MA; Lori Leibold, PhD; Sara Hansen, MS
Affiliations: Boys Town National Research Hospital, Center for Perception and Communication in Children

Background and Objective: In May 2022, Boys Town National Research Hospital's Center for Perception and Communication in Children, funded by a Phase II COBRE grant, acquired a custom mobile research lab, aptly named the Boys Town Research Vehicle (BTRV). We intend to use the BTRV to overcome barriers to participating in research, with the long-term goal of contributing to better health outcomes for more people, especially those in underserved communities. In this poster, we present the theory underlying our initiative to mobilize research and our plans for translating theory to action.
Methods: Our methods are informed by Donaldson's (2007) theory-driven program evaluation. We engaged in research, dialogue, and reflection to create a model of our program theory. We then shared this model with peers for refinement and validation.
Results: The primary result of this work is a visual model of our program theory with internal (from the program team) and external (from peers and literature) validity.
Discussion and Conclusions: By articulating our program theory, we can practice greater intentionality in our use of the BTRV, hold ourselves accountable to our goals, and evaluate our work to create a model that can be shared and replicated. Over the past year, we have established a solid foundation for our initiative to mobilize research through a cycle of experimentation, evaluation, and adaptation. We are now positioned to trial multi-day travel and data collection, community-research partnerships, and clinic-research partnerships with the BTRV. As we proceed, we will use the program theory as a foundation and a framework for evaluation and decision-making.
Acknowledgements: The BTRV was funded by the NIH under a Notice of Special Interest (NOSI): Administrative Supplements for Equipment Purchases for NIGMS-funded Center and Core Facilities (3 P20 GM109023-08S1). This presentation is supported with funds from the NIH under award number P20GM109023.

36. Flow Cytometry Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory.

Peter R. McDonald [1], Robin C. Orozco [1 2], and P. Scott Hefty [1 2].

Affiliations:
1 Flow Cytometry Core, Chemical Biology of Infectious Disease, The University of Kansas, Lawrence, KS, USA and **2** Department of Molecular Biosciences, The University of Kansas, Lawrence, KS, USA

Abstract:

The University of Kansas Flow Cytometry Core (KU-FC) provides access to flow cytometry and cell sorting instrumentation and expertise to researchers. Services and training are provided for flow cytometry: cell sorting and multi-parametric analysis of individual cells in solution, calculated from their fluorescent or light scattering characteristics. The KU-FC provides assistance in sample processing, data analysis, instrument training, software support, method and grant assistance, manuscript support, and consulting. The KU-FC is a 980 ft^2 BSL-2 facility equipped with a BD FACSymphony™ S6 Cell Sorter, a BD FACSAria™ Fusion cell sorter, a Cytek™ Aurora Spectral Flow Cytometer, and other supplemental assay instrumentation. The Cytek™ Aurora full-spectrum flow cytometry provides users with both tube-based and 96-well plate based spectral cytometry, with 5 lasers to allow analysis of 30+ colors. The BD FACS instruments allow measurement and sorting of up to 6 resolved populations of cells simultaneously, based on up to 50 parameters of detection using 18 simultaneous fluorochromes. The facility is equipped to handle BSL-2 samples and perform aseptic and single cell sorting into tubes or 96-well plates. The facility manages a FlowJo™ site license for data analysis software, and provides instrument training for users who desire to become self-operators of the facility instruments. The KU-FC will equip CBID researchers with tools directly applicable to infectious disease research, such as identifying and characterizing infectious agents such as bacteria and parasites, quantification and sorting of cells infected with microbial pathogens, and assessing chemical probe efficacy against infectious agents. The KU-FC resources enable monitoring immune responses and activation status associated with infection, and measuring changes in cellular phenotypes (size, granularity, complexity, density, expression) in response to compound treatment. The KU-FC seeks to assist CBID collaborators in achieving their research goals.

Scientific Focus Area: Core Facility

Grant Support:
P20 GM113117/NIGMS NIH HHS/United States Chemical Biology of Infectious Disease

37. Nebraska Center For Integrated Biomolecular Communication (NCIBC) Systems Biology Core Facility (SBC) – Molecular Analysis & Characterization Facility

Martha Morton,[1,2] Robert Powers,[1] Kurt Wulser,[1,2] Tom White,[2] Micah Jeppeson,[2] Jared Hass[1,2]

[1] Nebraska Center For Integrated Biomolecular Communication (NCIBC), University of Nebraska- Lincoln, Lincoln, NE 68588-0304

[2] Molecular Analysis and Characterization Facility, Department of Chemistry, University of Nebraska – Lincoln, Lincoln, NE 68588-0304

The NCIBC Systems Biology Core Facility (SBC) has been strategically designed to: 1. Enhance existing technology and enable the application of omics and instrumentation methodology among users. 2. Provide the necessary skills, tools, and training for systems biology analysis by investigators and other core facility users. Thus, SBC is expected to enhance infrastructure in an area of critical importance to NCIBC researchers requiring streamlined access to metabolomics, proteomics and microscopy instrumentation and methodology.

The SBC Facility provides analytical instrumentation and expert services to researchers from both academia and industry: 1. Life and food sciences (plant/ animal cells lysates, serum, urine, and micro-organisms such as bacteria, and yeasts. 2. Chemical and material sciences (such as synthetic and natural compounds, polymers, rare-earth materials, nanoparticles, and nanofibers. 3. Electrical engineering, chemical engineering, biomedical engineering, physics, and mechanical engineering. 4. Troubleshooting material defects.

The core has also been actively participating in undergraduate and graduate training and outreach education programs. The goal of NCIBC SBC is to enhance collaboration amongst faculty while broadening projects beyond standard ELISA methods or materials methods. Staff member train users but also collaborate on projects to enhance research and broaden research skills.

Acknowledgements: The Nebraska Center For Integrated Biomolecular Communication (NCIBC) is supported by NIH NIGMS P20 GM113126.

38. THE SYNTHETIC CHEMICAL BIOLOGY CORE (SCB): A RESOURCE FOR RESEARCH IN CHEMICAL BIOLOGY

Chamani T. Perera[1,2]

[1]Higuchi Bioscience Center, University of Kansas, Lawrence, KS, USA; [2]KU Synthetic Chemical Biology Core Laboratory, University of Kansas, Lawrence, KS, USA

The Synthetic Chemical Biology Core strives to provide comprehensive synthetic chemistry capabilities to investigators under one roof. The synthetic expertise of the core includes, but is not limited to, novel and commercially unavailable small molecules, fluorescent molecules and custom peptides. The core assists in identifying hits for medicinal chemistry optimization in infectious disease targets and provides synthesis capabilities for structure activity studies of said hits. The core staff will work with investigators to design and synthesis novel molecular probes to facilitate their research. SCB core encompasses the Purification and Analysis Laboratory (PAL) that provides purification, analysis and quality control of compounds via LC/MS. The SCB core also provides MALDI-TOF analysis of biomolecules.

39. Expansion of CryoEM in the Midwest: A New CryoEM facility has Emerged in the State of Nebraska.

Eduardo E. Romero[1,2,3]

[1]*Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE,68588, USA.*

[2]*Nebraska Center for Biotechnology, University of Nebraska-Lincoln, Lincoln, NE,68588, USA.*

[3]*Nebraska Center for Biomolecular Communication (CIBC), University of Nebraska-Lincoln, Lincoln, NE,68588, USA.*

CryoEM is one of the most relevant emerging tools for structural and cellular biology, due to its versatility and straightforward workflow. In the last couple of years, CryoEM has had important growth in the structural biology field, leading to near-atomic resolution structures competing and almost out-taking X-Ray crystallography. Consequently, the demand for CryoEM facilities in the US and worldwide have substantially increased as structural information can be obtained in hours to a few days, reducing the need for crystallizing proteins. Furthermore, despite the availability of multiple regional and national CryoEM facilities in the US, the Midwest is underserved and requires new CryoEM facilities with state-of-the-art instrumentation to produce high-quality research at low costs and at reasonable timelines. In this conference, we present the capabilities of our recently built CryoEM facility at the University of Nebraska-Lincoln with the support of the Nebraska Center for integrated biomolecular communication (CIBC) and the Nebraska Center for Biotechnology. The facility aims to serve the CryoEM research needs of the state of Nebraska and neighboring states and act as a bridge and point of contact to National centers for CryoEM. This work is supported by CIBC under the COBRE funded grant P20 GM113126, NIGMS.

40. Infectious Disease Assay Development Core: High Throughput Screening Laboratory at the University of Kansas

Anuradha Roy, PhD

IDAD-HTS Laboratory, University of Kansas, Lawrence, KS, USA

The overall goal of the IDAD Core is to provide expertise, facilities, services, and training in the area of HTS assay design, development, validation, small and large-scale screening for whole cell based or biochemical infectious disease targets. The IDAD core is an extension of the University of Kansas High Throughput Screening Laboratory which is a fee-for-service, state-of-the-art facility dedicated to providing academia, not-for-profit institutions, biotech, and pharmaceutical industries with exceptional assay development, high throughput screening and data mining services at economical rates. The staff has experience in executing cell-based, biochemical, siRNA as well as high content screening campaigns against a plethora of target classes. The laboratories are equipped with cutting-edge liquid handling and signal detection instrumentation for increasing throughput and precision of screening campaigns. Clients have the option of using our collection of 395,000 compounds and/or a client's own chemical library. KU-IDAD/HTS lab further leverages the strengths of the medicinal chemistry/ computational modeling cores under CoBRE Chemical Biology of Infectious diseases (CBID) program to support your tool/lead discovery research.

41. Supplementing Comparative Behavior and Cognition Data through Real-Time Physiological Assessment and Visualization in Small Rodent Models

Mystera M. Samuelson[1,2]

1. *Animal Behavior Core, Department of Comparative Medicine, The University of Nebraska Medical Center*
2. *Environmental, Agricultural, and Occupational Health Department, College of Public Health, The University of Nebraska Medical Center*

Abstract:

Background & Objective

Comparative behavior and cognition research relies heavily on ethological, ecological, and evolutionary research which has established and defined the innate behavioral repertoires and umwelts of small rodent species commonly used in laboratory studies. This has allowed for the development of specialized assays aimed at quantifying key behavioral and cognitive constructs. However, continued research in these foundational fields have revealed that these species are much more complex and diverse than originally thought. Still, our approach to the validation of novel assays and the assessment of novel rodent strains often remain unchanged. The purpose of this presentation is to present options for the validation of novel assays, and for defining the umwelt and behavioral repertoires of novel rodent strains.

Methods

Behavioral core facilities have a responsibility to support investigators by providing access to equipment and expertise, as well as instruction regarding best practices for data collection and interpretation. When working with novel rodent strains, best practices should include umwelt assessments, training in low-stress handling, environmental standardization, as well as the option to validate behavioral and cognitive studies with real time secondary measurements and analyses. To accomplish this aim, telemetric assessments, auditory and vestibular testing, bioacoustics, and gait analyses should be available alongside, and in conjunction with, traditional behavioral and cognitive assays.

Results

Resultant data from these assessments are leveraged to refine future studies to promote a cost and time effective investigation, which is both rigorous and easily reproducible.

Discussion & Conclusions

Proactive umwelt assessments should be considered an essential guide for any study involving novel rodent strains. Further, real-time physiological and bioacoustic assessments conducted during behavioral and cognitive assays will serve to groundtruth findings from traditional behavioral assays, as well as guide the refinement of these procedures.

Grant Support:

The Animal Behavior Core is supported by state funds from the Nebraska Research Initiative, and federal support from CDC-NIOSH U54OH010162-09, and the Cognitive Neuroscience of Development and Aging (CoNDA) through the Centers for Biomedical Research Excellence (COBRE) Program (NIH 1P20GM130447-01A1), as well as internal support from the Office of the Vice Chancellor for Research. **ABRF reference number RRID:SCR_018830**

42. The Auditory & Vestibular Technology Core: A COBRE-Supported Research Facility

Anthony S. Stender, Creighton University, Omaha, NE 68102 USA

The Auditory & Vestibular Technology Core (AVT) provides critical support for principal investigators and research teams associated with the Translational Hearing Center, thereby enabling them to conduct cutting-edge auditory and vestibular research across the full range of experimental model systems, from single molecule analysis to whole organism models. The AVT provides a broad range of technological tools and research services within the areas of electrophysiology, molecular biology, mass spectrometry, and advanced imaging. This facility is designed to enhance research efforts by assisting investigators in achievement of research goals, fostering collaboration, providing technical support to users, maintaining equipment, and continually striving to provide leading technology required of auditory and vestibular research. The facility operates under a hybrid fee-for-service model in which individual researchers are either trained to become advanced users of core resources or provided with specialized services by core facility employees.

The AVT Core Research Facility is located within the Creighton University School of Medicine and is supported by the Translational Hearing Center at Creighton University, Boys Town National Research Hospital and University of Nebraska Medical Center with CoBRE Award GM139762 from the National Institute of General Medical Science, a component of the National Institutes of Health. The content herein is solely the responsibility of the author(s) and does not necessarily represent the official views of any supporting institution.

43. The Integrated Biomedical Imaging Facility – A Light Microscopy Research Core

Anthony S. Stender, Creighton University, Omaha, NE 68102 USA

Research in the biomedical sciences often relies on imaging tools that provide high temporal and spatial resolution of tissues, cells, and dynamic processes; however, the high costs associated with such tools can put them out of reach for many investigators. The Integrated Biomedical Imaging Facility (IBIF) at Creighton University is committed to providing investigators with a diverse selection of advanced imaging tools and data analysis platforms. IBIF operates on a model where IBIF staff maintain instrumentation, provide hands-on training with the core's tools, and invoice investigators based on their monthly usage. IBIF staff are also available to consult on the design of experiments and data analysis.

IBIF's current resources consist of the following five light microscopes and two standalone data analysis workstations: 1) an upright Leica SP8 confocal and multiphoton laser scanning microscope with high-speed detectors for studies of fluorescence dynamics (FLIM, FCS, FRET); 2) an inverted Nikon Ti-E confocal microscope with a Yokogawa spinning disk that enables imaging in real-time at rates up to 1000 frames/s; 3) an inverted Nikon Ti-E widefield microscope for real-time imaging of ratiometric molecular probes or other fluorescent samples; 4) an inverted Total Internal Reflectance Fluorescence (TIRF) microscope for super-resolution, single molecule imaging; and 5) an inverted ImageXpress Micro 4 microscope from Molecular Devices for multi-day imaging experiments. Both the Nikon confocal and the ImageXpress offer incubation controls as well as automated imaging of well-plates. IBIF's two standalone data analysis workstations offer users a location to perform image analysis using the software packages supported by IBIF's microscope vendors: Leica, Nikon, and Molecular Devices.

IBIF is supported by Creighton University School of Medicine, and grants from the National Institute for General Medical Science (NIGMS): P20 GM103427 (INBRE), and P30 GM110768 (COBRE).

44. THE UNIVERSITY OF OKLAHOMA BIOMOLECULAR STRUCTURE CORE

Oklahoma COBRE in Structural Biology

Leonard M. Thomas[1]and Blaine H.M. Mooers[2]

[1]Department of Chemistry and Biochemistry

University of Oklahoma, Norman, OK 73019

[2]Department of Biochemistry and Molecular Biology

University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

The 3D structures of a protein of interest can answer current questions and suggest new hypotheses to understand structure-function relationships. In addition, the structures of protein-ligand complexes are critical to understanding key determinants of biomolecular recognition and for use in structure-based drug design. The Biomolecular Structure Core (BSC)* (https://www.ou.edu/structuralbiology/cobre-core-facilities) offers services and training to facilitate the structure determination of biomacromolecules. The services range from identifying initial crystallization conditions to structure determination, analysis, and preparation for publication. The BSC also provides services for the virtual screening of large drug libraries to narrow the search for new drug leads. The BSC interfaces with the high-throughput drug screening facility of the Oklahoma Center for Therapeutic Sciences to advance the project to the next level. The BSC can also collect small-angle X-ray scattering (SAXS) data.

The BSC can assist with the preparation of grids for Cryo-EM Single Particle Analysis (SPA). The facility can help with the shipping of the grids to national facilities for SPA data collection and with the transfer and processing of SPA data. A Tundra TEM for grid screening and collection of SPA data is scheduled for delivery to BSC-Norman and commissioning in November of 2023.

Each location of the BSC (OU-Norman and OUHSC) houses instrumentation for X-ray diffraction studies and Cryo-EM grid preparation. Crystallization robots support the high-throughput screening of crystallization conditions. A Formulatrix Rock Imager 1000 provides automated imaging of the crystallization experiments. Each location houses a Rigaku 007HF MicroMax X-ray generator to collect X-ray diffraction data. The BSC coordinates X-ray diffraction and SAXS data collection at the Stanford Synchrotron Radiation Lightsource 3 to 6 times a year. Computational resources are available for data processing, structure determination, structure analysis, virtual screening, and molecular graphics.

*The BSC is supported, in part, by COBRE grants P20GM103640 and P30GM145423

(PI: Ann West).

45. The Drug Discovery & Delivery Core in the Translational Hearing Center at Creighton University

Chunkai Wang[1], Alekha Dash[2], Sandor Lovas[1], Peter Steyger[1], Jian Zuo[1], Patrick Swanson[3], Gopal Jadhav[4]

Bellucci Translational Hearing Center, [1]Department of Biomedical Sciences, [2]Department of Pharmacy Sciences, [3]Department of Medical Microbiology and Immunology, [4]Department of Pharmacology and Neuroscience, Creighton University, Omaha, Nebraska, United States

Background and Objective

The Drug Discovery & Delivery Core (DDDC) at the Creighton Translational Hearing Center is establishing a cutting-edge drug development pipeline to facilitate individual research projects within and outside the Center. This includes a medicinal chemistry pipeline, high-throughput screens, novel drug delivery strategies to the inner ear with pharmacokinetic/pharmacodynamic methodologies, as well as creating a sustainability plan for DDDC services. With strong support from Creighton University and neighboring institutes, the DDDC has obtained the necessary equipment and expertise.

Methods

The DDDC uses modern, innovative, robust chemical synthesis and computational chemistry methodologies to systematically modify chemical structures for optimizing candidate ototherapeutic drugs' efficacy and bioavailability while minimizing toxicity. The DDDC also maintains highly efficient and accurate analytical services to provide greater confidence in the structural integrity, purity, and stability of requested molecular syntheses. Lead compounds undergo preformulation studies to yield better-characterized therapeutic candidates for further preclinical and clinical testing.

Results

The DDDC has completed multiple projects, including synthesizing compounds not commercially available (e.g., NBD-chloroquine conjugate), reference compounds (e.g., Compound 18 for hair cell regeneration), novel drug candidates (e.g., piperlongumine derivatives), as well as providing cost-effective solutions for high-cost commercial compounds (e.g., fluorescein−cisplatin conjugates) requested by Center investigators. In addition, DDDC has performed preformulation studies of two novel indole-2-carboxamindes against nontuberculous mycobacteria (Agrawal, et al., AAPS 2022) and has synthesized peptides using a microwave-assisted solid-phase peptide synthesizer.

Discussion and Conclusions

Identifying researchers with the necessary abilities to support large research investments is a major challenge for academia and industry worldwide. At the DDDC, we seek to ensure that the pipeline of graduate trainees understand the research problems in academia and industry. We see opportunities for both academic and for industry-academic engagement. We have established a track record of advancing drug discovery/synthesis and launching new research initiatives on time and within budget. We continue to expand our skillsets and to invest in a knowledge-based drug discovery core within a dedicated academic medical center.

The DDDC is supported by NIGMS CoBRE Award P20GM139762.

46. Title	Neuroimaging Acquisition and Analysis Core of the Cognitive Neuroscience of Development & Aging (CoNDA) Center
Authors	David E. Warren, Valentina Gumenyuk, James Brown, David Ellis, Anna Dunaevsky
Institution	University of Nebraska Medical Center (UNMC), Omaha, NE 68198
Scientific Focus	Core Facility

Abstract

1. Background and Objective: Cognitive neuroscience projects studying human subjects are increasingly reliant on advanced neuroimaging and neurostimulation methods. A key aim of UNMC's CoNDA Center is to support world-class neuroimaging and neurostimulation facilities and techniques for investigators in the eastern Nebraska region. The Center's Neuroimaging Acquisition and Analysis Core (NAAC) incorporates outstanding resources for cognitive neuroscientists who wish to use magnetic resonance imaging (MRI), magnetoencephalography (MEG), and/or transcranial magnetic stimulation (TMS) in pursuit of their research goals.

2. Methods: CoNDA's NAAC provides CoNDA and other regional investigators with access and support for neuroimaging and neurostimulation of human subjects with field-leading MRI, MEG, and TMS instruments and techniques. The Core's main instruments include: a Siemens 3T Prisma MRI system with advanced gradients, a complete suite of neuro MRI sequences, and multiple head coils (20-, 32-, and 64-channel); a MegIN Truix Neo MEG system with 306 channels (204 magnetometers and 102 gradiometers); and a Nexstim 5.1 Navigated Brain Stimulation TMS system with real-time stereotactic alignment of brain imaging data and participants' physical brains.

3. Results: Use of CoNDA's NAAC facilities has greatly increased and diversified since the Center's initiation. In addition to supporting CoNDA Research Project Leads, Pilot Projects Awardees, and Core Voucher recipients, the NAAC supports ongoing research, often NIH-funded, led by more than a dozen principal investigators from regional institutions. NIH-funded projects collecting data using NAAC facilities are sponsored by NIGMS, NIA, NIMH, and NCI. Additionally, the volume of NIH R01 applications from investigators utilizing CoNDA NAAC facilities has increased substantially since receiving the award.

4. Discussion and Conclusions: The UNMC CoNDA's NAAC has and will continue to enhance neuroimaging and neurostimulation in the service of advancing the cognitive neuroscience research of eastern Nebraska investigators. Acknowledgements/funding: UNMC's CoNDA Center is supported by an NIGMS P20 COBRE award (P20 GM130447).

47. Title: Compositional Analysis Methods for DNA Methylation based Deconvolution Estimates

Authors: Alexander M Alsup1, Devin Koestler1

Affiliations:

1. Department of Biostatistics & Data Science, University of Kansas Medical Center, Kansas City, KS

Background and Objective:

DNA methylation (DNAm) deconvolution provides a framework whereby cell-specific signatures of DNAm are leveraged to deconvolve the proportion of cells in a heterogeneous biospecimen. (e.g., whole blood). Due to the compositional nature of cell proportion data, use of traditional approaches, like linear regression, to examine the association between cell proportions and variable(s) of interest may lead to misleading or erroneous results. The goal of this work was to explore the application compositional analysis methods to cell proportion data and to benchmark the performance of such approaches against traditional methods.

Methods:

We conducted a series of simulation studies, generating cell counts for several cell types, of which a certain number of cell types were randomly selected to be differentially abundant between two groups. Cell counts were converted to cell proportions and used to conduct differential abundance tests using linear regression and compositional methods. Statistical power and False Discovery Rate (FDR) were calculated for each method and across each simulation scenario. We also conducted a simulation study where we assessed whether Neutropenia in one study group would skew the results of hypothesis tests on Eosinophil proportions when all other cell type abundances were consistent between groups.

Results:

The FDR for linear regression was ≥0.95 for sample sizes above 250, while FDR remained at or below 0.05 at N=1,000 for the compositional model. In assessing the effect of Neutropenia, the regression model falsely identified differential abundance of Eosinophils 94.2% of the time, while the compositional model did so 2% of the time. The compositional analysis method maintained a power above 0.90 at sample sizes above 125 in both simulations.

Discussion and Conclusions:

The results of our study indicate that traditional methods for analyzing cell proportion data can inflate false discoveries, whereas compositional methods provide adequate control of the FDR and maintain strong statistical power.

Focus Area: Data Science

Grant Support: N/A

48. Title: ***Simulating time-to-event data under the Cox proportional hazards model: assessing the performance of the nonparametric Flexible Hazards Method***

Short Title: ***Assessing the performance of the nonparametric Flexible Hazards Method***

Authors: Jennifer Delzeit[1] and Devin Koestler[1]*

Author Affiliations:

1. Department of Biostatistics & Data Science, University of Kansas Medical Center, Kansas City, KS

* To whom correspondence should be addressed.

ABSTRACT

Generating time-to-event data from a Cox proportional hazards (CPH) model is especially relevant in the context of biomedical research. While numerous methods and approaches have been developed for generating time-to-event data from the CPH model, they often require specification of a parametric distribution for the baseline hazard even though the CPH model itself makes no assumptions on the distribution of the baseline hazard. In line with the semi-parametric nature of the CPH model, a recently proposed method called the Flexible Hazards Method generates time-to-event data from a CPH model using a nonparametric baseline hazard function. While the initial results of this method are promising, it has not yet been comprehensively assessed with increasing covariates or against data generated under parametric baseline hazards. To fill this gap, we conducted a comprehensive study to benchmark the performance of the Flexible Hazards Method for generating data from a CPH model against methods that explicitly specify the distribution of the baseline hazard. Our results showed that with a single covariate, the bias in the Flexible Hazards Method is 0.02 (with respect to the log hazard ratio) with a 95% confidence interval having coverage of 84.4%. With 10 covariates, this bias increases to 0.054 and the coverage decreases to 46.7%. In this presentation, we explain the plausible reasons for this phenomenon as the number of covariates are increased, both empirically and theoretically, and provide readers and potential users of this method with some suggestions on how to best address these issues.

49. Title: Machine Learning for Image-Driven Quality Control in Bioprinting Applications

Authors: Samuel Haas, Timothy Hartman, Bichar Dip Shrestha Gurung, Evgeni Radichev, Gideon Kassa, Venkata Gadhamshetty, Etienne Z. Gnimpieba

Affiliations: Department of Biomedical Engineering, University of South Dakota, Sioux Falls; Department of Computer Science, University of South Dakota, Vermillion; Department of Material Engineering, Dartmouth College, Hanover, NH; Department of Civil and Environmental Engineering, South Dakota School of Mines and Technology, Rapid City

Background and Objective: Biomaterials in 3D structures mimic natural conditions better than those in 2D structures, which makes evolution in the realm of 3D scaffolds inevitable as bioprinting increases in clinical relevance. Additionally, leveraging bioprinting has become an important strategy in high throughput 3D experimental design toward high end application optimization, both in eukaryotic and prokaryotic 3D cell culture protocols. However, a lack of accessible semi-automatic parametrization of current technology remains a bottleneck in unlocking the full potential of bioprinting with regard to experimental design and better reproducibility.

Methods: We leverage semi-automatic bio-image acquisition and analysis using objective statistical methods and machine learning to minimize this limitation, both for more data capture, and to fine tune the bioprinter parameters for a better reproducibility. Our system was tested using Allevi bioprinting and Python for machine learning, image reconstruction, and data analysis.

Results: Our novel image analysis toolkit uses Cellpose machine learning-based segmentation and can provide quality control of image sets. The toolkit can be used before printing to assess material properties, during printing to assess layer quality, and after printing for fabrication quality and for cell seeding experiments.

Discussion and Conclusions: The proposed data driven bioprinting process enables a more accurate replication of biological conditions and leads to the discovery of new biomaterials and scaffold models. Researchers may pull from this discovered data to strengthen their experiments with better materials and 3D-printable scaffolds.

Acknowledgements:

- NSF RII Track-1: Building on The 2020 Vision: Expanding Research, Education and Innovation in South Dakota, Award # 1849206
- NSF RII Track-2 FEC: Data Driven Material Discovery Center for Bioengineering Innovation, Award # 1920954
- South Dakota Biomedical Research Infrastructure Network, NIH, Project # 5P20GM103443-20

50. Extreme QTL Mapping Reveals Zinc Toxicity Resistance Loci

Hanson, Katherine[1], Long, Anthony D.[2], and Macdonald, Stuart J.[1]
[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS
[2]Department of Ecology and Evolutionary Biology, University of California, Irvine, CA

Zinc is involved in many cellular functions, and toxicity can have widespread effects including necrosis, mitochondria inhibition, and homeostatic disruption of other heavy metals. The response to zinc toxicity is a complex trait, and our goal is to identify genes segregating for allelic variation for zinc resistance using an unbiased genomewide mapping approach. *Drosophila melanogaster* is an ideal model to study zinc resistance since homeostasis is regulated by evolutionarily conserved zinc transporter protein families, *MTF-1* (metal response transcription factor) and has proved to be a successful model understanding other heavy metal response traits. To identify zinc resistance loci, we employed extreme QTL mapping, a powerful technique that identifies QTL via selecting and sequencing pools of outbred individuals with extreme phenotypes. Our population was established by mixing DSPR (*Drosophila* Synthetic Population Resource) strains, a set of intercross lines derived from 8 inbred founder lines. We raised animals from this population on toxic $ZnCl_2$ media, sequencing 12 replicate pools of surviving, zinc resistant adult females, and a matching control pool. We estimated the founder composition from each pooled sample, and within the genome identified QTL as significant frequency shifts between control and selected pools. We identified 7 QTL, and for most only 1-2 founder alleles show a substantial frequency change between the control and selected populations, implying that highly resistant/susceptible alleles are rare. We identified 24 candidate genes and tested 18 via RNAi, with 11 being hits including *MTF-1*. Our work highlights several recognized, and novel contributors to metal metabolism in flies.

51. Title: Assessment of immune cell profiles among post-menopausal women in the Women's Health Initiative using DNA methylation-based methods

Authors: Emily Nissen[1], Alexander Reiner[2], Simin Liu[3], Robert B. Wallace[4], Annette M. Molinaro[5], Lucas A. Salas[6], Brock C. Christensen[6,7,8], John K. Wiencke[5,9], Devin C. Koestler[1*], Karl T. Kelsey[10*]

Affiliations:

[1]Department of Biostatistics & Data Science, University of Kansas Medical Center, Kansas City, KS

[2]Division of Public Health Science, Fred Hutchinson Cancer Center, Seattle, WA

[3]Departments of Epidemiology, Medicine, and Surgery, Brown University, Providence, RI

[4]Departments of Epidemiology and Internal Medicine, School of Public Health, University of Iowa, Iowa City, IA

[5]Department of Neurological Surgery, University of California San Francisco, San Francisco, CA

[6]Department of Epidemiology, Geisel School of Medicine, Dartmouth College, Lebanon, NH

[7]Department of Molecular and Systems Biology, Geisel School of Medicine, Dartmouth College, Lebanon, NH

[8]Department of Community and Family Medicine, Geisel School of Medicine, Dartmouth College, Lebanon, NH

[9]Institute for Human Genetics, University of California San Francisco, San Francisco, CA

[10]Departments of Epidemiology and Pathology and Laboratory Medicine, Brown University, Providence, RI

*authors contributed equally

Abstract:

Background: DNA-methylation (DNAm) based deconvolution methods leveraging cell-specific DNAm markers of immune cell types have been developed to provide accurate estimates of the proportions of leukocytes in peripheral blood. Immune cell phenotyping using DNAm markers, termed methylation cytometry, offers a solution for determining the body's immune cell landscape without requiring fresh blood and is scalable to large sample sizes. Despite significant advances in DNAm-based deconvolution, references at the population level are needed for clinical and research interpretation of these additional immune layers. Here we aim to provide references for immune populations in a group of post-menopausal American women.

Methods: We applied DNAm-based deconvolution to a large sample of post-menopausal women enrolled in the Women's Health Initiative (*N*=58) or its ancillary Long Life Study (*N*=1237) to determine reference ranges of 58 immune parameters, including proportions and absolute counts for 19 leukocyte subsets and 20 derived cell ratios. Further, we assessed the effect of age on immune cell parameters.

Results: Participants were 50-94 years old at the time of blood draw and we observed significant associations between age at blood draw and absolute counts and proportions of naïve B, memory CD4+, naïve CD4+, naïve CD8+, memory CD8+, neutrophils, and natural killer cells. We also assessed the same immune profiles in a subset of paired longitudinal samples collected 14-18 years apart. These results demonstrate high inter-individual variability in rates of change of leukocyte subsets over this time. When testing the difference in counts and proportions between these longitudinal samples there were significant changes in the same cell-types as the cross-sectional analysis, except for natural killer cells.

Conclusions: Here, we show these novel methods replicate known immune profiles associations with age and demonstrate the value this methylation cytometry approach has as it relates to establishing reference ranges and can add as a potential application in epidemiological studies.

Grant support:

The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through 75N92021D00001, 75N92021D00002, 75N92021D00003, 75N92021D00004, 75N92021D00005. Research was supported by the National Cancer Institute (NCI) Cancer Center Support Grant P30 CA168524; the Kansas IDeA Network of Biomedical Research Excellence Bioinformatics Core, supported by the National Institute of General Medical Science award P20 GM103418; the Kansas Institute for Precision Medicine COBRE, supported by the National Institute of General Medical Science award P20 GM130423; CA253976; the CDMRP/Department of Defense (W81XWH-20-1-0778); NIGMS (P20 GM104416); X01HL139376.

52. Leveraging High Performance Computing (HPC) Resources for Large Scale Analyses of Human Attention Data

Karissa Payne[1], Brian Howatt[1], Sahand Shaghaghi[2], Lester Loschky[1]; [1]Kansas State University, [2]University of Waterloo

Here we will discuss the analyses conducted for a recent study comparing human attention to video stimuli as measured by an eye tracker and a blur-based mouse cursor tracking paradigm we developed. In particular, we will discuss the role of the Kansas State University HPC cluster Beocat in running complex multilevel models on eye and mouse data for distance traveled per video frame (polled at 30Hz) and x, y coordinate similarity per frame for 27 video stimuli. HPC resources allow for multiple complex models to be run in parallel, allowing lengthy analysis plans on large-scale data to be completed in a fraction of the time. The featured analyses consist of multilevel General Linear Model (GLM) and Generalized Linear Model (GzLM) models run in R, alongside Bayesian multilevel GzLM models parallelized with between- and within-chain parallelization. Recommendations will be given for running similar models through the Open Science Grid.

Acknowledgements: This work was supported by the Cognitive and Neurobiological Approaches to Plasticity (CNAP) Center of Biomedical Research Excellence (COBRE) of the NIH under grant number P20GM113109.

53. Automating Tabulation of Peer-Reviewed Research Publications for the University of Kansas Cancer Center

Authors: Whitney Shae[1]; Devin Koestler[1]; Jeffrey Thompson[1]; Dinesh Pal Mudaranthakam[1]; James Dailey
[1]The University of Kansas Medical Center Department of Biostatistics & Data Science

Background and Objective
One measure of productivity for NCI-designated cancer centers is the quantity of peer-reviewed research publications within a defined time-period. Manual curation of publications is timing consuming, error prone, and undermines productivity. Though tools have been developed that semi-automate the process, there is a need for fully automated tools for the accurate tabulation of publications. Our aim is to provide this tool, first in service to the University of Kansas Cancer Center, with future applications specific to the goals of any research center.

Methods
Natural language processing (NLP) is a computer science method of learning and predicting the meaning of written text. We show that these methods can replace the manual curation of publications. Our methods begin with training an NLP model to detect cancer-relevance of publications using a portion of the data from previous years (training data) that had already been manually determined to be cancer relevant, or not. To assess our trained model's prediction ability, we apply the model to the remaining data that the model has not yet seen (test data), then compare our model's predictions of cancer relevance to the manual annotations.

Results
We completed this processing using the previously annotated abstracts of 411 KUCC-associated publications, reserving a test set of size n=80. Our model had performance metrics sensitivity=0.87 and specificity=0.88.

Discussion and Conclusions
Our results indicate that NLP is a promising method to automate the process of quantifying productivity for NCI-designated cancer centers. Next steps include optimizing performance and providing a user-friendly interface that be accessed by any member of the Cancer Center.

Research reported was supported by: the National Cancer Institute (NCI) Cancer Center Support Grant P30 CA168524; the Kansas IDeA Network of Biomedical Research Excellence Bioinformatics Core, supported by the National Institute of General Medical Science award P20 GM103418; the Kansas Institute for Precision Medicine COBRE, supported by the National Institute of General Medical Science award P20 GM130423.

Scientific Focus Area: Data Science

54. The genetic basis of adaptation to copper pollution in *Drosophila melanogaster*

Elizabeth R Everman[1,2], Stuart J Macdonald[1,3], John K Kelly[4]
[1]University of Kansas, Molecular Biosciences, Lawrence, KS, USA; [2]University of Oklahoma, Biology, Norman, OK, USA; [3]University of Kansas, Center for Computational Biology, Lawrence, KS, USA; [4]University of Kansas, Ecology and Evolutionary Biology, Lawrence, KS, USA

Heavy metal pollutants can have long lasting negative impacts on ecosystem health and can shape the evolution of species. The persistent and ubiquitous nature of heavy metal pollution provides an opportunity to characterize the genetic mechanisms that contribute to metal resistance in natural populations. We examined variation in resistance to copper, a common heavy metal contaminant, using wild collections of the model organism *Drosophila melanogaster*. Flies were collected from multiple sites that varied in copper contamination risk. We characterized phenotypic variation in copper resistance within and among populations using bulked segregant analysis to identify regions of the genome that contribute to copper resistance. Copper resistance varied among wild populations with a clear correspondence between resistance level and historical exposure to copper. We identified 288 SNPs distributed across the genome associated with copper resistance, many of which had population-specific effects. Significant SNPs mapped to several novel candidate genes involved in refolding disrupted proteins, energy production, and mitochondrial function. Our analysis revealed several novel candidate genes that may be involved in resistance to copper toxicity in addition to candidate genes that have been previously associated with copper resistance. Collectively, our results demonstrate that the genetic control of copper resistance is highly polygenic and that several loci can be clearly linked to genes involved in heavy metal toxicity response. A mixture of parallel and population-specific SNPs points to a complex interplay between genetic background and the selection regime that modifies the effects of genetic variation on copper resistance.

55. Title: AMEND: Active Module identification using Experimental data and Network Diffusion

Authors: Samuel S. Boyd[1,3], Chad Slawson[2,3,4], Jeffrey A. Thompson[1,3]

Author Affiliations:

1. Department of Biostatistics & Data Science, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66103 USA
2. Department of Biochemistry, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66103 USA
3. University of Kansas Cancer Center, Kansas City, KS, USA
4. University of Kansas Alzheimer's Disease Research Center, Fairway, KS, USA

Abstract:

Molecular interaction networks have become an important tool in providing context to the results of various omics experiments. A challenge is to determine, in the context of the interaction network, the subset(s) of genes that best captures the main mechanisms underlying the experimental conditions. One emerging area of interest is to determine which genes are equivalently or inversely changed between different experiments. The equivalent change index (ECI) is a recently proposed metric that measures the extent to which a gene is equivalently or inversely regulated between two experiments. The goal of this work is to develop an algorithm that makes use of the ECI and powerful network analysis techniques to identify a connected subset of genes that are highly relevant to the experimental conditions.

We developed a method called Active Module identification using Experimental data and Network Diffusion (AMEND). The AMEND algorithm is designed to find a subset of connected genes in a PPI network that have large experimental values. It makes use of random walk with restart to create gene weights, and a heuristic solution to the Maximum-weight Connected Subgraph problem using these weights. This is performed iteratively until an optimal subnetwork is found. AMEND was compared to two current methods, NetCore and DOMINO, using two gene expression datasets.

The AMEND algorithm is an effective and easy-to-use method for identifying network-based active modules. It returned connected subnetworks with the largest median ECI by magnitude, capturing distinct but related functional groups of genes.

56. β_2-adrenergic receptor activation ameliorates neonatal lung immunity to Respiratory Syncytial Virus infection

Sandeep Adhikari[1], Pankaj Baral[1*]

Corresponding Author: baral@ksu.edu

[1]Division of Biology, Kansas State University, Manhattan, KS, USA, 66506.

Background and Objective: Respiratory Syncytial Virus (RSV) is the most common cause of acute bronchiolitis and pneumonia in children accounting for 33 million cases annually. RSV is a single-stranded RNA virus recognized by endosomal TLR7 and TLR8. There is no effective antiviral drug or vaccine for RSV. The respiratory tract is heavily innervated by sympathetic neurons that release the neurotransmitter noradrenaline which acts on β-adrenergic receptors for bronchodilation and immunomodulation. Exploring neuroimmune regulation strategies could provide insights for new therapeutics. Mining of single-cell RNA sequencing database demonstrates the elevated expression of β-adrenergic receptors mainly by macrophages and monocytes. β-agonists, albuterol and xamoterol activate β_1AR and β_2AR respectively. Albuterol is an FDA-approved drug for asthma and COPD. We hypothesize that β_2-adrenergic receptors, and treatment of neonates with their agonists, mediate antiviral immunity and host defense to RSV.

Method: C57BL/6J bone marrow-derived macrophages (BMDMs) were stimulated with R848, a TLR 7/8 agonist, in the presence or absence of β-adrenergic agonists. Alternatively, β-adrenergic knockout BMDMs were stimulated with R848. One-week old C57BL/6J were intranasally inoculated with RSV-A2 to induce neonatal pneumonia and later treated with multiple doses of albuterol to determine its therapeutic efficacy.

Results: *In-vitro* stimulation of BMDMs with R848 in the presence of albuterol suppressed pro-inflammatory cytokine TNF-α and upregulated anti-inflammatory cytokine IL-10. Opposing results were observed when we used BMDMs from β_2-adrenergic receptor knockout mice. I*n-vivo* data demonstrated marked improvement of lung damage in albuterol treated neonates and suppressed B-cells, $CD8^+$ T-cells to basal level in the bronchoalveolar lavage (BAL) fluids.

Discussion & Conclusion: β_2-adrenergic receptor activation can modulate cytokines induction to resolve lung pathology through suppression of recruitment of inflammatory cells during RSV infection. Overall, our data suggests the protective role of β_2-adrenergic receptor in regulation of airway inflammation and inflammatory cell response during RSV infection in neonates.

Keywords: Sympathetic neuron, Albuterol, β_2-adrenergic, RSV-A2, Immune cell

Acknowledgement: This work was funded by K-INBRE P20 GM103418. We extend our thanks to all our lab members for the support.

57. NOVEL APPROACH FOR SIMULTANEOUSLY QUANTIFYING HUMAN NATURAL KILLER CELL-MEDIATED ADCC AND DIRECT KILLING

Cami Bisson[1], Angela Truong[1], Maia M.C. Bennett[1], Alexander K. Regan[1], Bella Circo[1], Arriana Blackmon[1], Isabelle Weber[1], DJ Rogers[1], Anna R. Mahr[1], Paul W. Denton, PhD[1]
1 - Department of Biology, University of Nebraska at Omaha, Omaha, NE

Background and Objective

Immunotherapies have revolutionized patient care approaches in many disease contexts, including in the treatment of malignancies as well as infections. Importantly, a primary objective of immunotherapy is improving immune-mediated clearance of diseased cells. However, different cells of the immune system may respond to immunotherapies differently depending on the cell type and/or the context of immune function. Natural killer (NK) cells are effector immune cells which destroy their targets using one of two methods: direct killing or antibody-dependent cellular cytotoxicity (ADCC). In the case of direct killing, NK cells can recognize a missing receptor on the target cell and initiate an apoptotic response. In ADCC, an antibody bridges the target and the NK cell facilitating a cytotoxic response.

Methods

Our lab has developed a testing system, referred to as the Natural Killer – Simultaneous ADCC and Direct Killing Assay (NK-SADKA), that allows for the analyses of both NK cell-mediated cytotoxic outcomes using cells from a single human donor. Human target cells (e.g., Daudis and K562s) are labeled with a fluorescent dye to distinguish them from NK effector cells. Target and effector cells are combined and co-incubated [with an antibody known to facilitate ADCC (e.g., Rituximab) added to the ADCC arm]. Target killing is quantified using flow cytometry.

Results

Optimized key experimental conditions are to co-incubate effector and target cells at an E:T ratio of 5:1 for 2 hours. This was true for both direct killing and ADCC. Importantly, we found that individual human donors can routinely be evaluated in both the direct killing and the ADCC arms of the NK-SADKA.

Discussion and Conclusions

We will present our data that confirm the functionality of the NK-SADKA as a reliable and reproducible strategy for testing the impacts of immunotherapies on both NK cell direct killing as well as ADCC functions. (Supported by INBRE: P20 GM103427)

58. Distribution of PERV (Porcine Endogenous Retrovirus)-C among Feral Pigs in Eastern Kansas

Cesur, Robin. Gillock, Eric. Fort Hays State University, Hays, KS

Background and Objective

Allotransplantation (using human organs for transplant) can sometimes be arduous in the United Stated due to a lack of donors. Xenotransplantation can be used to help alleviate these shortages. Pigs are an animal that may be used for this. Pigs (wild and domestic) can be a problem when it comes to Porcine Endogenous Retrovirus (PERV). This is a retrovirus that can become introduced into the germ line of a pig. Infectious viral particles have been released in some immortalized pig cell lines. Since retroviruses have been known to cause lymphomas and leukemias, this could potentially be a problem. There are three subtypes based on cell tropism, sequence variation, and receptor interference; A, B, and C. A and B are present in all pigs and infects pigs and humans. C is only present in some pigs and only infects pigs. The goal of this study is to see how prevalent this virus is among a feral population in eastern Kansas.

Methods

This study is using DNA extraction, PCR, and gel electrophoresis to detect the presence of PERV-C in a sample size of 53 in Eastern Kansas.

Results

It was found that 44/53 (83%) were positive for PERV-C, 13/31 (42%) were positive for PERV A/C hybrid long. The short variety is still being examined.

Discussion and Conclusion

This knowledge will aide in understanding how prevalent this subtype is in the feral population. This will also guide further studies in learning structure and function in retroviruses and their related family members (koala retrovirus, murine leukemia virus, and feline leukemia virus.

Affiliations: Fort Hays State University, Biology Department

Grant support: K-INBRE Grant P20 GM103418

59. Antiseptic Polycationic Triazolium Salts: Structure-Activity Relationship Study

James T. Fletcher, Adam J. Burr, Laura M. Cogua

Department of Chemistry and Biochemistry, Creighton University, Omaha, NE 68178

Background and Objective

Antimicrobial resistant pathogens remain an urgent threat to public health. Quaternary ammonium compound (QAC) antiseptics are commonly used tools for sanitizing surfaces, but their prevalent usage has resulted in the evolution of QAC-induced bacterial resistance. Hence, there is an urgent need for developing next-generation antiseptic compounds that remain potent against microbial targets but are structurally unique relative to commercial QACs such as benzalkonium chloride. The goal of this study was to prepare 1,3,4-trisubstituted-1,2,3-triazolium salts and evaluate how structural identity impacts antiseptic properties, including the impact of installing multiple cationic centers within the same compound.

Methods

Target compounds were prepared using a two-step synthetic sequence of tandem Click reactions to make 1,3- and 1,4-disubstituted-1,2,3-triazole compounds, followed by N-benzylation reactions to make 1,3,4-trisubstituted-1,2,3-triazolium salts. Minimum inhibitory concentration assays were used to evaluate antimicrobial properties against Gram-positive bacteria, Gram-negative bacteria and yeast BSL-1 model organisms. Select compounds from these assays were additionally evaluated for potency against clinically relevant and drug-resistant Gram-negative bacteria.

Results

Target compounds were successfully prepared in moderate to high yields and good purity. No differences in synthetic efficiency were observed between preparing monocationic and polycationic triazolium salts. The most potent analogs displayed minimum inhibitory concentration values as low as 1 micromolar against Gram-positive bacteria, 4 micromolar against Gram-negative bacteria and 4 micromolar against yeast. Monocationic compounds were generally less potent than polycationic compounds.

Discussion and Conclusions

The modular nature of preparing 1,3,4-trisubstituted-1,2,3-triazolium salts allowed efficient preparation of analogs enabling structure-activity relationship profiling of antiseptic properties to be completed. Polycationic triazolium salts generally display significantly increased antiseptic potency relative to monocationic analogs. Antiseptic potency against clinically relevant Gram-negative bacteria was generally consistent with that of BSL-1 model Gram-negative model organisms, supporting the initial screening of BSL-1 organisms to identify compounds potentially useful to combat clinically relevant pathogens.

Acknowledgements

This publication was made possible by grants from the National Institute for General Medical Science (NIGMS) (5P20GM103427), a component of the National Institutes of Health (NIH), and its contents are the sole responsibility of the authors and do not necessarily represent the official views of NIGMS or NIH.

60. Discovery of Immunomodulatory Agents for GI Immunity

Grahmm A. Funk[1], Anuradha Roy[2], Hyunjoon Kim[1]

[1]Department of Pharmaceutical Chemistry, University of Kansas

[2]COBRE CBID Infectious Disease Assay Development Core, University of Kansas

Background and Objective: Immunotherapy serves to modulate the host immune system to fight foreign pathogens or suppress autoimmunity. Conventional immunotherapies utilize parenteral injections, with limited utilization of oral delivery (e.g., polio vaccine). Additionally, traditional therapies induce systemic humoral immune response (IgG), but are less effective at inducing mucosal immune responses (IgA), which are critical to clear pathogens and reduce autoimmune activation in the lung and GI tract. This study aims to discover and validate new compounds capable of modulating mucosal immunity and to create a vaccine delivery platform for efficient oral delivery.
Methods: High-throughput screening (HTS) of two drug libraries including FDA-approved compounds was used to identify initial "hit" molecules. Screening was done with RAW 264.7 Blue cells, utilizing a RAW-NF-kB promotor-SEAP (Secreted embryonic alkaline phosphatase) assay in 384-well plates. SEAP is used as a reporter to measure NF-kb activation to identify potential immune modulators. Quantification of SEAP in each well at 620nm was measured and compared to DMSO controls.
Results: Initial screening yielded a total of 18 "hit" compounds that gave a substantial increase in SEAP over DMSO controls (3-4x). In addition, several other compounds showed significant reductions in SEAP absorbance.
Discussion and Conclusions: Initial discovery of potential new immunomodulatory drugs is crucial to develop increasingly potent and targeted immunotherapies. With early candidates identified, we may now proceed with further *in vitro* characterizations using immune cells (e.g., BMDC, macrophages) and toxicity assessment. Upon completion of hit compound validation, we will develop oral vaccine delivery platforms using nano/microparticles and hydrogels.
Acknowledgement: This work was supported in part by a grant from CoBRE-Chemical Biology of Infectious Disease (NIH award number P20GM113117) (H.K). Personnel funding (G.A.F) provided by the Madison and Lila Self Graduate Fellowship.

61. Characterizing High Persister Phenotypes in *Staphylococcus epidermidis* Clinical Isolates

Mariam Garcia[1], Kaitlyn Pineda[1], and Austin Nuxoll[1]
[1]Department of Biology, University of Nebraska at Kearney, Kearney, Nebraska

Background and Objective

Staphylococcus epidermidis is an opportunistic pathogen that typically resides within our normal skin flora and is primarily associated with causing disease in immunocompromised individuals. Often these infections are biofilm-mediated and associated with indwelling medical devices. Antibiotic treatment of these infections is often unsuccessful, leading to poor patient prognosis. One possible explanation for these observations is the presence of persister cells (a subpopulation of dormant cells). High persister isolates have been observed in other microbial pathogens such as *Pseudomonas aeruginosa* and *Candida albicans*. Recent work in the related pathogen, *S. aureus*, demonstrates that persister formation is dependent on energy depletion through the tricarboxylic acid (TCA) cycle. We hypothesized that high persister isolates occur in
S. epidermidis clinical isolates through an energy-dependent mechanism.

Methods

To observe the possibility of a correlation between high persister formation and a dysfunctional TCA cycle, membrane potential was quantitatively measured through flow cytometry. Membrane potential was measured from different *S. epidermidis* strains at stationary phase. These strains were stained with DiOC2(3) for 30 minutes prior to FACS analysis. Carbonyl cyanide m- chlorophenylhydrazone (CCCP) was utilized as a control for gating cells with low membrane potential.

Results

Of the five isolates screened, two correlated with decreased membrane potential. Meanwhile, one correlated with increased membrane potential and had decreased persister formation. Preliminary data has demonstrated the correlational relationship between a dysfunctional TCA cycle and increased persister formation.

Discussion and Conclusions

S. epidermidis isolates exhibited varying degrees of tolerance, despite their similar MIC values to vancomycin. Furthermore, extracellular acetate concentrations didn't correlate with persister cell formation, and preliminary data indicate that flow cytometry in conjunction with membrane potential is a better indicator of persister activity in clinical isolates.

Acknowledgements

Research presented was supported by the National Institute of General Medical Science of the National Institutes of Health (NIH) under award number GM103427.

Scientific Focus Area: Infectious Disease and Immunology

62. *Chlamydia trachomatis* Inclusion Membrane Protein CT226 Interaction with Host Proteins TMOD3 and FLII.

Tanisha Goyal *[1], *Christina Bourne*[2], *Erika Lutter*[1]

[1]Oklahoma State University, Stillwater, OK, USA

[2]Oklahoma University, Norman, OK, USA

Background and Objective- *Chlamydia trachomatis* is an obligate intracellular pathogen that is a global health problem and the most reported bacterial sexually transmitted disease in United States. It can cause long term consequences such as pelvic inflammatory disease, ectopic pregnancy, and reduced fertility. Bacteria has a distinctive biphasic life cycle, its reproductive stage creates a parasitophorous vacuole, also known as an inclusion. *Chlamydia* produces inclusion membrane proteins which decorate the inclusion and mediate host-pathogen interactions. We hypothesize that Inc CT226 is interacting with host proteins FLII and TMOD3 at the leucine rich regions and verify this interaction using the bacterial two hybrid system (BAC2H) and pulldowns. Purified proteins of predicted interacting regions will be isolated and used for structural analysis, binding assays, and crystallography.

Methods- Using PCR we amplified the TMOD3 and FLII full length protein and leucine rich regions using specific primers. This was followed by digestion of the amplicon and the vector using appropriate restriction enzymes at 37° for four hours. The digested products were ligated and then transformed in DH5α and BL21 cells. The transformation product was plated on LB agar plates with antibiotics. The clones for TMOD3 transformed in BL21 cells were induced to enhance protein production, which was run on a 10% SDS-PAGE gel. For BAC2H we double transformed bait and prey plasmids into BTH101 cells and plated them on MacConkey agar plates with appropriate selection.

Results- Clones for TMOD3 and FLII (full length and leucine rich regions) in their suitable vectors and were confirmed by sequencing. The estimated size of the protein for TMOD3 was confirmed using an SDS-PAGE gel. Color change observed on MacConkey agar with double transformants in BAC2H system.

Discussion and Conclusions- CT226 interacts with full length of TMOD3 using BAC2H. TMOD3 protein products were produced successfully. We are working on verifying the interaction of both full length and leucine rich regions of FLII and leucine rich region of TMOD3 using BAC2H. The BAC2H results will be confirmed with β-galactosidase assays. Induced proteins will be purified and used for crystallography.

63. Response of type 2 innate lymphoid cells to peanut is sensitive to sex-specific differences

Alethia Henderson[1], Leigh-Anne Lehmann[1], Sunanda Rajput[1], McKenna S. Vininski[1], Nicholas J. Hobbs Ph.D.[1], and Joseph J. Dolence Ph.D.[1]
[1]Department of Biology, University of Nebraska at Kearney, Kearney, NE

Background and Objective:

An understanding of the mechanism in which peanut (PN) initiates immune responses to generate PN allergy remains limited. Specifically, how sex differences impact the development of PN-specific immune responses is unknown. This study compared male, female, and androgen receptor-deficient Tfm mice exposed to PN, via inhalation, in a 3-day mouse model to investigate how sex differences impacted the response of lung type 2 innate lymphoid cells (ILC2s).

Methods:

After 3-day exposure, lungs were collected and processed with a lung dissociation kit to obtain immune cells from the lungs to further analyze. Cells were stained with antibodies to identify ILC2s by flow cytometry.

Results:

We found ILC2s were sensitive to sex differences with ILC2s in female PN-exposed lungs having a more abundant response than ILC2s in male PN-exposed lungs. Tfm mice displayed a greater ILC2 response to PN compared to both male and female

wild type mice. We have shown that IL-1α is released by lung epithelial cells following inhalation of PN. Therefore, we wanted to examine whether ILC2s expressed IL-1R1, the receptor for IL-1α, and if these cells were sensitive to hormonal regulation. We discovered that ILC2s, especially KLRG1+ ILC2s, express IL-1R1 in response to PN, indicating ILC2s directly respond to IL-1α released by lung epithelial cells after PN inhalation. Furthermore, Tfm mice showed more severe ILC2 responses.

Discussion and Conclusions:

Taken together, this data suggests that sex hormonal differences between males and females influence the initial immune responses to peanut following inhalation of the allergen. The response we observed in the Tfm mice reveals that androgen sex hormones are important in regulating ILC2 responses to PN. Future studies will further elucidate the ILC2 populations to better understand how they are activated against PN with a particular focus for how sex differences impact these responses.

Dr. Dolence is supported by NE-INBRE which is funded by a grant from the National Institute of General Medical Science (NIGMS) of the National Institutes of Health (NIH) under award number GM103427

64. Neuroimmune regulation of carbapenem-resistant *Klebsiella pneumoniae* lung infection

Prabhu R. Joshi[1], Pankaj Baral[1]

[11]Division of Biology, Kansas State University, Manhattan, KS, USA, 66506.

Abstract

Background and objective

The respiratory tract is innervated by nociceptive sensory neurons (nociceptors), which recognize and respond to different environmental dangers and mediate pain as a defense mechanism. Pathogens represent another source of danger and the role of nociceptors to respiratory pathogens is less understood. The objective of this study is to determine the role of nociceptors and their secreted neuropeptide calcitonin gene-related peptide (CGRP) in lung defense to CRKP pneumonia.

Methods

Nociceptors-ablated and control mice were infected with lethal dose of carbapenem-resistant *Klebsiella pneumoniae* (CRKP). Bronchoalveolar lavage fluid (BALF), spleen and liver tissues were collected 24 hours post-infection. The bacterial load was measured by diluting and plating BALF/tissue lysate on Luria-Bertani agar. Immune cells in BALF were characterized by flow cytometry. Inflammatory cytokines in BALF were measured by enzyme-linked immunosorbent assay (ELISA). Neutrophils and monocytes were depleted using anti-Ly6G antibody and anti-GR1 antibody respectively to determine their roles in CRKP infection. Monocytes, CRKP and CGRP (1 μM) were co-cultured to determine the effect of CGRP on intracellular bacterial killing ability of monocytes.

Results

Ablation of nociceptors led to decreased bacterial load and trans-alveolar dissemination of bacteria following CRKP infection in mice. Furthermore, nociceptors suppressed the recruitment of neutrophils ($CD11b^{+}Ly6G^{+}$) and inflammatory monocytes ($CD11b^{+}Ly6C^{hi}$), and cytokine induction. Depletion of monocytes, but not neutrophils, abrogated the increased lung bacterial clearance and dissemination in ablated mice. Co-culture of monocytes and CRKP with nociceptive neuropeptide CGRP revealed the suppression of monocyte-mediated intracellular killing of CRKP *in vitro*.

Discussion and conclusion

Our *in vivo* and *in vitro* data demonstrate the host deleterious effects of nociceptive neurons and CGRP signaling for the anti-bacterial immune responses to CRKP infections. Data also suggests that the monocytes are critical cellular population downstream of nociceptors.

Key words: neuroimmune, nociceptive neuron, CGRP, CRKP.

Acknowledgements

We acknowledge funding sources CBID-CoBRE and K-INBRE for their support to our work.

65. Role of Nociceptive Neurons in Antifungal Immunity during *A. fumigatus* Lung Infection

Chinemerem Onah[1], Michael Bartkoski[1], Pankaj Baral[1]

[1]Division of Biology, Kansas State University, Manhattan Kansas 66506

Abstract

Background and Objective: The immunocompromised individuals are more likely to acquire Invasive Pulmonary Aspergillosis (IPA) and lethal pneumonia caused by *Aspergillus fumigatus*. IPA and fungal pneumonia cause dysregulated inflammation, altered immune responses, and rapid destruction of airway barrier. The bronchioles and airways are heavily innervated by nociceptive neurons, a major subset of sensory nervous system, that help maintain homeostasis and immune cell functions. Although *A. fumigatus*-specific therapeutics exist, they fail to provide absolute protection against IPA and comes with substantial clinical limitations. Therefore, it is important to understand the crosstalk between lung-innervating neurons and the immune system to identify novel anti-fungal therapeutic target(s) by understanding the signaling pathways between these two systems.

Methods: We crossed *Trpv1-Cre* and *LoxP-DTA* mice to generate Trpv1-Cre$^{+/-}$/DTA$^{+/-}$ (nociceptive neuron-ablated) and Trpv1-Cre$^{-/-}$/DTA$^{+/-}$ (non-ablated littermate control) mice. Then we intranasally infected these mice with 10^8 *A. fumigatus* conidia per mouse. Bronchoalveolar lavage (BAL) fluid and whole lungs were harvested at 24 hours and analyzed for fungal burden, immune cell recruitment, and cytokine/chemokine levels.

Results: Our data indicate an increased fungal burden in the airspaces and whole lung among the nociceptive neuron-ablated mice than the control mice. We also noticed extensive immune cell recruitment especially neutrophils in the absence of nociceptive neurons.

Discussion and Conclusions: Altogether, our data support the notion that nociceptive neurons and play a crucial role in immune cell recruitment and anti-fungal immunity during *A. fumigatus* infection. Further research is necessary to better understand the link between the anti-fungal immune mechanism and nociceptive neuron signaling for finding the mechanism of therapeutic drug targets.

Acknowledgement: Kansas Idea Network of Biomedical Research Excellence (K-INBRE), Johnson Cancer Research Center, Kansas State University.

66. Growing Antibiotic Resistance in the European honey bee (Apis mellifera) Microbiome

Destiny Brockhaus[1] and Kayla I. Miller[1]

1: Dakota Wesleyan University, Mitchell, SD

Background and Objectives

Honey bees play a crucial role in agriculture and are suffering dramatic losses. One emerging area of research into these losses examines the role of the microbiome in the health of bees. The microbiome plays vital roles in health and conversely, disruption of the microbiome has been correlated with disease states. The honey bee is a heavily managed agricultural insect and is prophylactically treated against bacterial bee pathogens with antibiotics. This extensive use of antibiotics has also resulted in extensive antibiotic resistance in the microbiome. As a result, new antibiotics have been approved for use in honey bee hives, however, the level of resistance to these antibiotics has not been measured. This research investigates the amount of antibiotic resistance to antibiotics within the honey bee gut microbiome.

Methods

Bacterial isolates from the honey bee intestinal tract were tested against commonly used antibiotics used in agriculturally managed hives: oxytetracycline and tylosin tartrate. A standard assay to calculate the minimum inhibitory concentration (MIC) for each isolate against each antibiotic was used to determine and compare their level of resistance.

Results

Our results suggest that bacteria from the honey bee intestinal tract are becoming less resistant to the antibiotic oxytetracycline, which previously had been the dominant antibiotic used in managed honey bee populations from the 1970s to 2010s. However, we also see that isolates are rapidly developing resistance to the newest antibiotic being used in honey bee hives, tylosin tartrate, which was approved in 2005.

Discussion and Conclusions

Extensive use of antibiotics for prophylaxis is increasing the amount of antibiotic resistance in the honey bee microbiome. This has implications not only for the health of the honey bee and the ability to manage diseased hives, but also for the growing global concern of widespread antibiotic resistance.

67. Skeletal Muscle Oxygen Delivery and Uptake in Cardiopulmonary Diseases

Hannah F. Wall[3], Kiana M. Schulze[1,2], Ramona E. Weber[1,2], Andrew G. Horn[1,2], K. Sue Hageman[1,2], David C. Poole[1,2], Timothy I. Musch[1,2]

Department of Kinesiology, Kansas State University[1], Department of Anatomy and Physiology, Kansas State University[2], Department of Biology, Langston University[3], Kansas State University, Manhattan Kansas, Langston University, Langston Oklahoma

Abstract

Introduction: Pulmonary Hypertension (PH) is a life-threatening lung disease, causing hypertrophy of pulmonary arteries. Heart Failure (HF) occurs following a myocardial infarction (MI), where part of the heart dies and no longer functions properly. Both of these pathologies decrease patients' quality of life and exercise capacity. In this study, we assess how PH and HF affect skeletal muscle oxygenation and therefore, function. We hypothesized that skeletal muscle oxygenation will be decreased in PH and HF rats compared to healthy control (HC) rats, measured as the partial pressure of oxygen in the interstitial space (PO_2*is* (dependent upon $\dot{Q}O_2$ (blood flow)/$\dot{V}O_2$ (mitochondrial oxygen utilization)). **Methods:** Sprague-Dawley rats were randomized into monocrotaline-induced PH, MI-induced HF, or healthy control groups. Each group's PO_2*is* was measured using the O_2 sensitive probe Oxyphor G4 in the left spinotrapezius muscle at rest and during electrically induced contractions. **Results:** We found that PO_2*is* is lower (all comparisons $P<0.05$) in both disease groups at rest (PH HC = 21.4±2.3, HF HC = 25.5±1.0, PH = 15.1±1.9, and HF = 21.4±1.8) and during contractions (PH HC = 11.0±1.4, HF HC = 15.1±1.4, PH = 8.4±0.9, and HF = 11.5±0.5). **Conclusion:** These findings demonstrate that skeletal muscle PO_2*is* is decreased in PH and HF rats compared to healthy counterparts, providing one mechanism for decreased exercise capacity in these diseases. Future studies will aim to understand individual contributions of $\dot{Q}O2$ and $\dot{V}O2$ to the reduced $PO_2$is and develop effective countermeasures.

68. Neural mechanisms of biased attention towards disorder-salient stimuli in binge-type eating disorders

[1,2]Jeffrey S. Johnson, [2]Ross D. Crosby, [2]Stephen J. Wonderlich

1. North Dakota State University, Fargo, ND
2. Sanford Center for Biobehavioral Research, Sanford Health, Fargo, ND

Binge-eating disorder (BED) is a serious medical condition characterized by recurrent episodes of binge eating, in the absence of extreme compensatory behaviors (American Psychiatric Association, 2000). BED is associated with high rates of psychiatric and medical comorbidity, including obesity. Research has suggested that binge eating and related behaviors may be promoted and/or maintained by excess attention to environmental sources of information related to weight, shape, and food. However, the temporal dynamics of attentional engagement, the specificity of attention biases towards disorder-salient stimuli, and the underlying neural mechanisms that may give rise to them are still poorly understood. Although recent years have witnessed a marked increase in basic neurocognitive research related to eating disorders, it is generally acknowledged that the field lags behind other psychiatric disorders in terms of progress in understanding the brain circuits and pathophysiology of this debilitating form of mental illness. Gaining this understanding is critical if the promise of refined treatment targeting BED and related disorders are to be realized. For this presentation, we will describe a COBRE-funded study that uses innovative behavioral and electrophysiological methods to measure attention biases towards disorder-salient stimuli, state-of-the-art neuroimaging methods to assess brain functional connectivity, and correlation-based statistical approaches to examine the relationship between the two. This convergent approach makes it possible to test specific hypotheses regarding core processes that may be disrupted in binge-type eating disorders. Basic knowledge about the neural basis of attention biases in this population can provide insights into the nature of a large number of psychiatric and neurological disorders that feature maladaptive preoccupations with disorder-relevant stimuli, such as anxiety, mood, and substance use disorders. Moreover, understanding attention biases and their neural bases can contribute to the development of novel behavioral and brain stimulation-based methods of effectively treating these debilitating conditions.

This research is funded by grant P20 GM134969-03 awarded to SJW

69. *Drosophila* Tak1, Tab2, and MyoVI function in protein autophagy

Biven, Erica,[1] and Geisbrecht, Erika R. [1]

[1]Department of Biochemistry and Molecular Biophysics, Kansas State University

Background and Objectives:

Maintaining the three-dimensional structure of proteins is crucial for proper function. Misfolded or denatured proteins must be restored to their original folded shape or degraded. For normal protein turnover, the correct cellular machinery must be recruited to degrade target proteins in the proteasome or by autophagy in the lysosome. When disrupted, damaged proteins accumulate, and cellular or organismal death can occur. In autophagic protein clearance in *Drosophila melanogaster*, a complex of the S/T kinase NUAK and Starvin (Stv), the ortholog of the mammalian Bcl-2-associated athanogene 3 (BAG3), cooperate to recruit components of the autophagosome, including p62 [1]. The human homolog, NUAK1, belongs to a family of kinases known to be phosphorylated by four different upstream kinases: Liver kinase B1 (Lkb1), Ca2+/calmodulin-dependent PK kinase β (CaMKK), transforming growth factor-β-activated kinase 1 (Tak1), and S/T kinase 38 (STK38) [2,3].

Methods:

Levels of candidate kinases were decreased in muscle tissue using RNA interference (RNAi). After dissection, *Drosophila* larval tissue was stained with antibodies, imaged using a confocal microscope, and analyzed for the abnormal accumulation of proteins and/or autophagic structures.

Results:

Muscle defects due to protein accumulation in *Tak1 RNAi* knockdown is exacerbated in a *NUAK+/-* heterozygous background. No significant defects resulted from knockdown of Lkb1, CaMKK, or the *Drosophila* homolog of STK38, called Tricornered (Trc). Tab2 is known to mediate the activation of Tak1 [4]. Tab2 knockdown produced muscle defects that were enhanced in a *NUAK+/-* background, indicating Tak1 and Tab2 may function in the same pathway. Knockdown of another protein implicated in autophagy, Myosin VI (MyoVI), causes mild muscle defects and increased p62 levels, both of which are exacerbated in a *stv+/-* background.

Discussion and Conclusions:

These results together suggest Tak1, Tab2, and MyoVI function with the NUAK-Stv complex to regulate either protein and/or mitochondrial autophagy in muscle tissue.

Grant Support:

Funding for this research was provided by NIH RO1 AR060788 and an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20 GM103418.

70. Title: Sleep impairment among adults with recurrent binge eating

Authors: Leah Irish,[1,2] Kristine Steffen,[2,3] Ross Crosby,[2] Scott Engel,[2] Kathy Lancaster[2] & Stephen Wonderlich[2]

[1]Department of Psychology, North Dakota State University, Fargo, ND
[2]Sanford Center for Biobehavioral Research, Sanford Research – North, Fargo, ND
[3]Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND

Abstract:
Background: Poor sleep is a prospective risk factor for obesity, but little is known about the extent to which disordered eating behaviors, such as binge eating, may serve as mechanisms linking sleep to weight outcomes. This ongoing CoBRE project investigates associations between sleep, binge eating, and weight gain across six months. We present preliminary descriptive analyses from our baseline data to characterize the presence of sleep problems among binge eaters.
Method: To date, baseline data have been collected from 74 adults who engage in recurrent binge eating (i.e., at least one episode per week for the past four weeks). Binge eating frequency was evaluated by clinical assessors using the Eating Disorder Examination interview. Sleep was assessed for two weeks using wrist actigraphy and sleep diary. In addition, participants reported their perceptions of sleep using the Sleep Perceptions and Attitudes Questionnaire.
Results: More than half of the sample of binge eaters experienced significant actigraphy-assessed impairments in sleep duration and sleep efficiency. Several problematic beliefs and attitudes toward sleep were also common in the study sample.
Conclusions: Preliminary findings from our baseline data confirm the existence of objectively verified sleep disturbance among adults who engage in recurrent binge eating. Longitudinal data collection for this project is ongoing and will allow for further testing of the potential mediating role of binge eating in the prospective association between poor sleep and weight-related outcomes. Sleep has great potential as a novel target in the treatment of eating disorders and obesity.
Acknowledgements: Funding for this presentation and ongoing project provided by the National Institutes of Health (P20 GM134969) Center for Biobehavioral Mechanisms of Eating Behavior (PI: Wonderlich/PD: Irish).

Scientific focus area: Other – behavioral health

71. The Molecular and Electrical Signal Regulation of the Migration of Dental Pulp Stem Cell Derived Chondrocytes

Audrey Scherrman, Nikol Flynn, Pranita Kaphle, Li Yao

Department of Biological Sciences, Wichita State University, KS

1. Background and Objective

Human dental pulp stem cells (HDPSCs) are attractive stem cells for cartilage and nucleus pulposus (NP) generation. The cells are easily accessible and have demonstrated similar differentiation capability as other MSCs. The transplantation of hDPSCs-derived chondrogenic cells encapsulated in type II collagen hydrogels mimicking the native cartilage and NP tissue is potentially a novel approach to regenerating the degenerated cartilage and NP. In a previous study, we reported the differentiation of hDPSCs toward chondrogenic cells and cell culture in the hydrogels. The motility of transplanted cells is critical because the cells need to migrate out of the hydrogels and disperse through the tissue. We reported the differentiated cell migration in the type II collagen gels. In degenerated cartilage and discs, the expression of cytokines such as IL-1β and TNFα increased significantly compared with healthy cartilage and NP tissue. Electric fields have been identified in developing and wounded tissue. In this study, we investigated effect of these factors on cell migration.

2. Methods

HDPSCs are differentiated into chondrogenic cells and grown in type II collagen hydrogels. The migration of cells subjected to IL-1β and TNFα treatment was recorded with time-lapse imaging. The cultured differentiated cells were treated with electric fields and the migration direction was determined.

3. Results

We show that the negative effect of IL-1β and TNFα on differentiated cell migration in the hydrogels. We also observed the guidance of electric signal to the differentiated cells.

4. Discussion and Conclusions

The study implicated a role of electric fields in tissue repair and the potential application in cartilaginous tissue regeneration.

Acknowledgements:

We would like to thank the K-INBRE grant # P20 GM103418 for their support of our research.

72. 2023 Central Region IDeA Conference
Poster Abstract

Title: Momentary bio-behavioral predictors of loss of control eating and weight outcomes: A CoBRE Project overview

Authors: Gail A. Kerver[1,2], Scott G. Engel[1,2], David B. Sarwer[3], Kristine J. Steffen[1,4], Ross D. Crosby[1,2], Kathryn Lancaster[1], Stephen A. Wonderlich[1,2]

Affiliations: [1]Sanford Center for Biobehavioral Research; [2]University of North Dakota School of Medicine and Health Sciences; [3]Temple University College of Public Health; [4]North Dakota State University Department of Pharmaceutical Sciences

Scientific Focus Area: Allied Health

Background and Objective: Loss of control (LOC) eating negatively impacts weight loss efforts among individuals with obesity. Bariatric surgery is the most effective treatment for obesity. However, LOC eating postoperatively has been consistently linked to poor weight outcomes. Biobehavioral mechanisms of post-surgical LOC eating are not well elucidated. Self-control (i.e., inhibitory control) is believed to be the mechanism through which negative affect (NA) prompts LOC eating. While traditionally assessed with patient-reported outcome measures, this mechanistic process has yet to be examined in real-time in the natural environment of bariatric surgery patients. Additionally, there is accumulating evidence that low blood glucose (i.e., hypoglycemia) may impact the relationship between inhibitory control and LOC eating. Therefore, the proposed project seeks to test the prospective relationships between NA, inhibitory control, hypoglycemia, and LOC eating in real-time and examine their collective contribution on longitudinal weight outcomes following bariatric surgery.

Methods: 50 adults who underwent a Roux-en-Y Gastric Bypass (RYGB) surgery one year prior to study enrollment will complete assessments at three timepoints. At baseline, participants will complete a series of self-report questionnaires and interviews and then engage in 10 days of Ecological Momentary Assessment (EMA), assessing emotional states and eating behavior. They will also provide 10 days of passive, continuous glucose monitoring to assess the frequency of hypoglycemia. Six- and 12-months later, participants will return to the lab to have their height and weight recorded in order to assess weight change. To date, 31 participants have been enrolled into the study protocol. It is anticipated that data collection will be completed by October 2024.

Significance/Impact: Results of this study have the potential to inform treatment intervention efforts aimed at reducing LOC eating postoperatively and promote more optimal weight outcomes.

Acknowledgement: This project is supported by grant P20GM134969 funded by the National Institute of General Medical Sciences.

73. Malvolio, the *Drosophila* Ortholog of Human NRAMP2 Metal Ion Transporter, is Required for Cell Morphogenesis

Authors:

Rajprasad Loganathan[1,2], Srihitha Akula[1], Tony Zhu[1], Aditi Kulkarni[1], Rika Maruyama[1], Deborah Andrew[1].

Affiliations:

1. Department of Cell Biology, Johns Hopkins University School of Medicine
2. Present: Department of Biological Sciences, Wichita State University

Background and Objective

Malvolio (Mvl) is a member of the SLC11 family of metal ion transporters and is the Drosophila ortholog of the mammalian natural resistance-associated macrophage proteins (NRAMPs). Mvl is implicated in the maintenance of cellular iron homeostasis. Whether Mvl has any roles in cell morphogenesis, independent of its homeostatic roles, is unknown. We are investigating the role(s) for Mvl in cell morphogenesis in the Drosophila embryonic salivary gland (SG).

Methods

We combined genetic analyses with imaging approaches to study the role(s) of Mvl in cell morphogenesis. We generated a null allele of *Mvl* (*Mvl^{exc1}*), transgenic fly lines for expressing both GFP-tagged and untagged Mvl (UAS-Mvl, UAS-Mvl-GFP), as well as Mvl-specific polyclonal antisera.

Results

Our studies show that Mvl expression is Fork head-dependent for SG morphogenesis. Our initial analysis showed no effect on viability in *Mvl* zygotic loss although developmental delay was observed in *Mvl^{exc1}/Df(Mvl)*. Zygotic loss of *Mvl* resulted in mild SG morphological defects and small gaps in the denticles. Combined maternal and zygotic loss, however, resulted in pronounced defects in SG morphology and increases in the frequency of the denticle gaps. The levels of the apical polarity determinant Crumbs were notably decreased with Mvl loss. Mvl also localizes to Golgi, early and late endosomes. Collectively, these results demonstrate varying degrees of cell morphogenetic defects with the loss of *Mvl* during embryogenesis.

Discussion and Conclusions

Our preliminary results lead us to hypothesize that the cell morphogenetic defects in *Mvl* loss of function are the likely result of defective endomembrane trafficking that affects Crumbs localization and recycling at the sub-apical domain.

Scientific Focus Area: Other (Cell and Developmental Biology)

Grant Support:

This study was supported by the National Institutes of Health grant R01DE013899 (DA).

74. Title: A Longitudinal Examination of Reward, Eating Expectancies, and Inhibitory Control in the Progression of Loss of Control Eating

Authors: Lauren M. Schaefer[1,2], Ross D. Crosby[1,2], Scott G. Engel[1,2], Kristie Stefffen[1,3], & Stephen A. Wonderlich[1,2]

Affiliations: [1]Sanford Center for Biobehavioral Research, [2]Unviersity of North Dakota School of Medicine and Health Sciences, [3]North Dakota State University

Background and Significance: Binge eating (BE) is a transdiagnostic symptom of multiple eating disorders, and is associated with significant medical and psychiatric comorbidity. Current interventions for BE are limited in their efficacy, suggesting the need for improved prevention and treatment approaches that directly target mechanisms of BE onset and maintenance. Theory suggests that positive eating expectancies (i.e., beliefs about the reinforcing consequences of food consumption) may be a key mechanism relating a history of reinforcement from eating and risk for BE symptoms. However, limited research has examined the hypothesis. Further, evidence suggests that abnormalities in reward processing and inhibitory control may compound risk for BE. However, no study to date has examined the prospective relationships between these constructs within a comprehensive theoretical model using multi-modal assessment. Therefore, this study will examine an etiological model of BE onset, which incorporates observed abnormalities in reward processing and inhibitory control within an expectancy-based framework among individuals demonstrating loss of control eating (LOC, a common precursor to BE) using a longitudinal design. **Method:** The project will test a model hypothesizing that greater reinforcement from LOC eating episodes at baseline (i.e., reductions in negative affect and increases in positive affect assessed using ecological momentary assessment) will lead to increases in eating expectancies at 3-month follow-up (i.e., expectancies that eating improves affect), which will subsequently lead to BE onset at 6-month follow-up. It is further hypothesized that higher reward responsiveness (e.g., reward sensitivity) will amplify the relationship between eating reinforcement history and eating expectancies, while decreased inhibitory control is hypothesized to amplify the relationship between eating expectancies and BE onset. **Potential Implications:** Given this project's focus on identifying mechanisms of BE symptom onset, findings from the current project would help to identify malleable risk factors for BE, which could be targeted in intervention efforts.

Grant Support: This work was supported by a grant from the National Center of General Medical Sciences (1PGM134969).

75. INVESTIGATING THE IMPACT OF MYOSTATIN ON DIABETIC RETINOPATHY

***Myka Heimbach*[1]**, *Hanna Russel[1], Kenzie Bethel[1], Melissa Vera[1], Dr. Joshua Butcher[2,3] and Dr. Cammi Valdez[1,3]*

[1]Natural Sciences, Northeastern State University, Tahlequah, Oklahoma.

[2]Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University

[3]Harold Hamm Diabetes Center, University of Oklahoma Health Sciences Center

Background: Diabetes is a highly prevalent disease in the U.S., effecting 34.2 million people. Diabetes can lead to many complications, including diabetic retinopathy, which causes microvascular degradation in the retina, ultimately leading to blindness. Myostatin, a negative regulator of skeletal muscle growth is overexpressed in diabetics, triggering oxidant stress. Oxidant stress is the leading cause of cell death in the microvasculature. However, when myostatin is deleted, vasodilation of the microvasculature is improved. We aim to investigate the effects of myostatin in the microvasculature of the retina *in vitro* and *in vivo* to better understand the development of diabetic retinopathy.

Methods: For our *in vitro* study, we transformed *E. coli* cells with a control construct and a construct containing myostatin to multiply the gene. We then extracted, purified, and linearized the control and myostatin construct DNA. Next, we transfected primary human retinal endothelial cells and began characterization. For our *in vivo* study, we used diabetic and diabetic myostatin knockout mice. We quantified retinal microvascular cells. Then, we characterized the retinas for acellular capillaries and microaneurysms.

Results: We have developed a stable primary cell line that overexpresses myostatin. Further analysis and characterization of the cells is ongoing. Microaneurysms were present in diabetic mouse retinas, but not diabetic myostatin knockout mice. Acellular capillaries were markedly present in diabetic animals and were less severe in diabetic myostatin knockout animals. Quantification retinal microvascular cells is still underway.

Conclusion: Our *in vivo* work shows myostatin deletion improves diabetic retinopathy disease progression. However, the mechanism for which myostatin plays in development of diabetic retinopathy is still unknown. Therefore, the use of our stable cell line overexpressing myostatin will be advantageous to elucidating the role of myostatin in diabetic retinopathy.

Grant Support: OK-INBRE Collaborative Grant through the National Institute of General Medical Sciences of the National Institutes of Health under award P20GM103447. The content in this publication is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

76. DEVELOPING AND CHARACTERIZING A NEW MOUSE MODEL OF DIABETIC RETINOPATHY

Cammi Valdez[1,2], Erica Dotson[1], Joshua Butcher[2,3], and Phillip Coburn[4]

[1]*Department of Natural Sciences, Northeastern State University*

[2]*Harold Hamm Diabetes Center, The University of Oklahoma Health Sciences Center*

[3]*Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University*

[4]*Department of Ophthalmology, The University of Oklahoma Health Sciences Center*

Background and Objective: As a common complication, 35% of all diabetic patients will develop some form of diabetic retinopathy and one-third of those patients will develop debilitating visual impairment or blindness. It has long been accepted in the field that hyperglycemic conditions lead to the microvascular degeneration that causes diabetic retinopathy. Although fructose has been associated with hyperglycemia, it is understudied and poorly defined mechanistically with regards to diabetes, despite it being highly prevalent in Western diet. Increased fructose consumption has been associated with higher risk of diabetic retinopathy in humans. However, the mechanism by which fructosemia aids in the progression of diabetic retinopathy is still poorly understood. Therefore, we propose to test the role and mechanistic pathways that drive retinal dysfunction in a diet-induced fructosemia mouse model.

Methods: Mice were fed control and high fructose diet ad libitum. Weights, non-fasting and fasting blood glucose, and A1C levels were measured every 3 months post-diet induction to assess metabolic health. Funduscopy, fluorescence angiography, and optical coherence tomography were performed to assess *in vivo* eye health. At 6-months post-diet induction, an insulin tolerance test and fluorescein dextran heart perfusions were performed.

Results: Fructosemia mice gain significantly more weight that control mice at 3-, 6-, and 9-months post-diet induction. Insulin tolerance tests at 6-months post-diet induction revealed insulin insensitivity in fructosemia mice. At 6-months post-diet induction, fructosemia model have increased retinal vascular permeability. At 9-months post-diet induction, fructosemia mice have increased fasting blood glucose and A1C levels compared to control.

Discussion and Conclusions: Our data suggests that fructosemia mice are developing metabolic syndrome and the retinal microvasculature is weakening at 6-months post-diet induction. Therefore, this new mouse model of fructosemia may prove promising for studying diabetic retinopathy in a more physiologically relevant system than those currently available.

Grant Support: OK-INBRE Research Project Investigator Grant through the National Institute of General Medical Sciences of the National Institutes of Health under award P20GM103447. The content in this publication is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

77. Impact of Simulated Galactic Cosmic Radiation and Hindlimb Unloading on the Mouse Adrenal Gland Morphology and Histology

Authors: Payton Nies[1], Shun'nya C. Taylor[1], Avanelle Stoltz[1], Makaila Olson[2], Marissa R Burke[3], Amber M Paul[3], Candice Tahimic[4], April E Ronca[5], Lane K Christenson[1]

Dept. Cell Biology and Physiology, University of Kansas Medical Center[1], Weill Cornell Graduate School of Medical Sciences[5], Embry-Riddle Aeronautical University[3], University of North Florida[4], NASA Ames Research Center[5]

Galactic cosmic radiation (GCR) combined with microgravity are concerns for astronauts going beyond the protective Van Allen belts for lunar and Martian trips. As the central endocrine tissue involved in stress management, the adrenal glands, are likely to play a critical role in responses to these spaceflight environmental stressors. The adrenal produces corticosterone, aldosterone, and epinephrine/norepinephrine to impact acutely and chronically cellular and tissue metabolism and glucose and sodium balances. In this study, we exposed adult (6 m old) female (n=18) and male (n=18) C57BL/6J mice to hindlimb unloading (HU) and 15 GrY (lunar) and 50 GrY (martian) simulated GCR irradiation (IR). Treatment groups included: HU, IR, control, HU+IR, group house control, and group house IR and animals were treated for 14 days prior to euthanasia and tissue collection. Adrenals were dissected and fixed for 24 h in 4% paraformaldehyde, followed by transfer to 70% EtOH, and shipment to KUMC. Adrenals were then embedded in paraffin and serial sectioned and 4 central sections from each were stained with H&E, prior to imaging on a Nikon 80i microscope. Whole adrenal cross section area, medullary area, and cortex area were determined. General adrenal gland morphology and histology did not appear to be affected by hindlimb unloading or GCR exposure. Total cross- sectional area, medullary region, and cortex of the adrenal were not different across treatment groups. Male adrenal cross-sectional areas are half the size of the female adrenals, due to a difference in cortex area. In conclusion, simulated microgravity and GCR did not dramatically affect the adrenal gland histology or size. Ongoing studies are evaluating functional corticosterone, aldosterone, and epinephrine tissue levels in these murine tissues.

Support from Accelerate Cancer Education Internship Program (KUCC), KINBRE Summer Scholars Program (KUMC) Grant P20 GM103418, NASA Human Research Program (HRP) Human Factors Behavioral Performance Element Grant 18 18FLAG 2 0028.

78. Treatment of renal progenitor cells with the proteasomal inhibitor Bortezomib reduces the expression of CD133, CD24 and the proximal tubule marker AQP1 and upregulates the expression of the distal tubule marker CALB1.

Rayna Rice, Kaija Berwald, Becker Linder, Danping Guo, Sarmad Al-Marsoummi and Scott H. Garrett. Department of Pathology, University of North Dakota, School of Medicine and Health Sciences, Grand Forks, North Dakota.

Background and Objective. Progenitor cells in the kidney have high regenerative capacity and the potential to repair damage due to injury. However, the mechanism involved in the regeneration of the tubules by the progenitor cells is not well understood. Previously, we have isolated progenitor cells (HRTPT) from the immortalized human proximal tubular cell line RPTEC/TERT1. These cells co-express CD133 and CD24 on their cell surface and can undergo neurogenic, adipogenic, osteogenic, and tubulogenic differentiation when cultured in specialized cell culture media. The proteasomal inhibitor Bortezomib is currently being used to treat patients with multiple myeloma and it can cause acute kidney injury. The goal of the current study is to determine if treatment of the renal progenitor cells with Bortezomid would affect the regenerative capacity of these cells.
Methods. The HRTPT cells were treated with 100nM and 200nM of Bortezomib for 48 hours. RNA and protein was extracted from the treated cells and RT-qPCR and Western analysis was performed to determine the expression of CD133, CD24, Aquaporin1 (AQP1), a water transporting protein on the cell membrane of proximal tubule cells of the kidney and calbindin 1 (CALB1), a calcium binding protein expressed on the surface of distal tubule cells of the kidney.
Results. Our data shows that inhibition of proteasomal activity by Bortezomid decreases the expression of CD133 and CD24 in the HRTPT cells. Furthermore, it also decreases the expression of AQP1 and increases the expression of CALB1 in these cells.
Conclusions. In conclusion, our study suggests that Bortezomid treatment can reduce the progenitor function of the HRTPT cells and reduce their ability to help regenerate the kidney following renal damage. The reduced expression of AQP1, a protein known to play a role in the migration the proximal tubule cells and the increased expression of CALB1 suggests that Bortezomid may be causing the progenitor cells to differentiate, thus reducing their regenerative capacity.

79. Treatment of renal progenitor cells with the proteasomal inhibitor Bortezomib reduces the expression of genes involved in FGF signaling.

Brooke Rossow, Kaija Kinnumen, Kaitlyn Berwald, Danping Guo, Sarmad Al Marsoummi and Scott H. Garrett. Department of Pathology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, North Dakota.

Background and Objective. The proximal tubule of the kidney is the major site of reabsorption of essential nutrients and accumulation of toxins from the bloodstream. When damage occurs to the kidney, fibroblast growth factors (FGF) are produced and signaling via the FGFR is induced and there is recruitment of progenitor cells to the site of new nephron development. This lab has previously isolated progenitor cells from the immortalized renal proximal tubular cell line, RPTEC/TERT1. These progenitor cells (HRTPT) express CD133 and CD24 and can differentiate into various cell types when cultured in specialized growth media. Gene expression and proteomic analysis has demonstrated that these cells express high levels of FGFR1, FGFR2, FGF2 and FGF9. Additionally, they express high levels of proteasomal proteins. Bortezomib is a proteasomal inhibitor that is used for the treatment of multiple myeloma and can cause acute kidney injury. The objective of this study is to understand the effect of proteasomal inhibition on FGFR/FGF pathway in renal progenitor cells which could potentially help to determine the role of the pathway in renal damage and repair.
Methods. The HRTPT cell line was treated with various concentrations of Bortezomib and the expression levels of FGFR1, FGFR2, FGF2 and FGF9 was determined by RT-qPCR and Western blot analysis.
Results. Treatment with Bortezomib significantly decreased the expression of FGFR1, FGFR2 and FGF9 in the renal progenitor cells whereas the expression levels of FGF2 was significantly increased.
Conclusion. Our study shows that treatment with the proteasomal inhibitor Bortezomib effects the FGFR signaling pathway which may reduce the ability of the renal progenitor cells to form new nephrons leading to acute and chronic kidney damage.

80. Thermoneutral housing produces greater increases in energy expenditure during wheel running in mice

Michael E. Ponte, John P. Thyfault, E. Matthew Morris

Background: Voluntary wheel running (VWR) is used to increase mouse physical activity, and hypothetically energy expenditure (EE). However, this may be confounded by the observed ~2-fold increase in mouse resting EE at standard, sub-thermoneutral housing temperatures. Herein, we used 20ºC & 30ºC housing temperatures to investigate the impact of divergent baseline EE on the capacity of VWR to change EE and mediate metabolic phenotypes. **Methods:** We performed indirect calorimetry experiments in male C57Bl/6J mice housed at 20ºC or 30ºC with VWR or without (SED) to assess total EE and the components of EE during 7-day low-fat (LFD) and high-fat, high-sucrose (HFHS) diets. **Results:** As expected, LFD mice housed at 30ºC have ~40% lower total EE and energy intake, and 60% lower resting EE compared to 20ºC mice. Interestingly, activity EE was ~30% greater in 30ºC mice for both VWR & SED. Importantly, while total activity was increased in VWR, no difference in total activity was observed due to temperature within SED or VWR. VWR increased total EE compared to SED regardless of temperature. VWR did not alter resting EE, which represented ~70% and ~50% of total EE in 20ºC & 30ºC mice, respectively. VWR increased activity EE ~55% at both temperatures. However, activity EE represented greater than twice the percent of total EE in 30ºC mice. During subsequent HFHS feeding, 7-day weight gain was reduced ~40% in male VWR mice regardless of temperature, with VWR having no impact on female diet-induced weight gain. **Conclusions:** While VWR reduced HFHS-induced weight gain at both temperatures, 30ºC mice had a greater VWR-mediated increase in total EE, absolute activity EE and as a percent of total EE. These data suggest that thermoneutral housing is more appropriate for studying the impact of VWR, and increases in EE, on metabolic disease outcomes.

81. EXPLORATION OF NUTRITIONAL AND ANTI-NUTRITIONAL COMPONENTS OF REDBUD SEEDS

Author(s): **Sergio A. Vazquez Gomez,** Asuncion Eleazar Rubio, Cooper McKinney, Mackenzie Powell, Skylar Fletcher and Dr. Nancy L. Paiva
University of Scholar: Southeastern Oklahoma State University, Durant, OK, USA
Location of Research: Southeastern Oklahoma State University, Durant, OK, USA
Mentor(s): Dr. Nancy L. Paiva, Southeastern Oklahoma State University, Durant, OK, USA.

Background & Objectives: Redbud trees (*Cercis canadensis*) are small leguminous trees native to North America. Centuries ago, Native American tribes consumed roasted immature redbud seeds and raw redbud flowers. However, little chemical research has been done to assess the nutritional value. **Methods, Results & Discussion**: Redbud seeds were examined for the accumulation of nutritional proteins, the accumulation of beneficial condensed tannins and presence of anthocyanidin reductase (ANR, a tannin pathway enzyme), and heat-sensitive growth-inhibiting substances. Immature redbud seeds were excised from developing pods and immediately frozen. Seed proteins were extracted and resolved on SDS-PAGE gels. Gels stained directly revealed four major protein bands (60, 35, 18 and 17 kDa) in mature redbud seeds, but these do not begin to accumulate until late June, and increase in intensity as seeds increase in volume. Gels were electro-blotted to PVDF membranes and processed with custom-developed anti-ANR serum; immuno-reactive bands were visible in late June harvests but the bands are smaller than expected, suggesting ANR protein was present but degraded. Amino acid analysis indicates significant levels of essential amino acids, except methionine. To test for growth-inhibiting substances, the growth of tobacco hornworms (*Manduca sexta*) was compared on media supplemented with cooked and uncooked ground redbud seeds, both mature and immature (green). Addition of uncooked green or uncooked mature redbud seed powder at 20%w/w significantly inhibited growth of young hornworms, suggesting that unheated seeds are toxic or unpalatable. Cooking green seed paste at 100ºC for 30 minutes greatly improved the growth of worms to near control levels in some trials. This suggests that roasting green seeds may reduce the toxicity of the seeds, as is required for many common edible legume seeds containing toxic lectins.
Funding: National Institutes of Health NIGMS award P20GM103447 for OK-INBRE, plus NASA Oklahoma Space Grant Consortium, and NSF-OK-LSAMP.

82. PROTEIN AND NATURAL PRODUCT CHARACTERIZATION OF REDBUD TREES

Cooper McKinney[1], *Asuncion Eleazar Rubio[1], Mackenzie Powell[1], and Nancy Paiva[1]*

[1]Department of Chemistry, Computer, and Physical Science, Southeastern Oklahoma State University, Durant, OK

Background and Objectives: Redbud trees (*Cercis canadensis*) are small leguminous trees native to North America. Indigenous Native Americans reportedly consumed roasted immature redbud seeds and raw redbud flowers. Bark and wood extracts were used as remedies for sore throats and congestion. However, little chemical research has been reported regarding phytochemical activities or nutritional value of redbud seeds. Our objective was redbud seeds for essential amino acids and total protein content, and bark and wood for natural products.

Methods: Oven-dried immature beans were powdered and processed by 3 methods to measure all 20 amino acids and total protein. SDS-PAGE was used to characterize protein banding patterns, for comparison to commercial nutritional legume seeds. Acetone extracts of plant parts were examined using TLC and agar-diffusion bioassays.

Results: Redbud seeds contained 15-16%(w/w dry weight) total protein, and were rich in essential amino acids, including lysine, as is typical for common edible beans. Highly abundant protein bands accumulate late in seed development, similar to patterns observed for common edible legumes. TLC data indicates fluorescent bands present in the heartwood.

Discussion and Conclusions: Amino acid profiles confirm that immature redbud seeds should provide a good source of essential amino acids. Protein banding patterns in maturing redbud seeds indicate that these may be seed storage proteins derived from one precursor protein; MALDI-TOF and sequencing analysis of gel-purified bands is planned. TLC bands indicate polar, fluorescent molecules. Bioassay is ongoing for wood components. GC-MS analysis is ongoing.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award P20GM103447. The content in this publication is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

976846309
566603143
157633264
090060986
713475982
473996521
749022360
702994087
209636637
711717255

83. Exercise Neuroprotection in a Model of Alzheimer's Disease

Veith, Sabrina, Kansas State University Department of Biology, White, Zachary, Department of Anatomy and Physiology, Sudasinghe, Keshari, Department of Anatomy and Physiology,

Rogers, Liza, Department of Kinesiology, Hall, Stephanie, Department of Anatomy and Physiology

BACKGROUND AND OBJECTIVE: Alzheimer's disease (AD) is one of the most devastating age-related diseases for which there is no cure; however, exercise has shown promise as a therapeutic method.[1–5] The present study aims to evaluate the effect of aerobic exercise on body mass, spatial memory and learning, coordination, and muscular strength in rat model of AD. **METHODS**: A novel rat model of AD, TgF344-AD, were bred and housed two per cage on a 12:12 light:dark cycle with food and water *ad libitum*. At 3 weeks of age, animals were genotyped and placed in either a wild type control group (WT) or Alzheimer's disease group (AD). Additionally, animals were assigned to either sedentary (SED) or exercise training (EX) groups. At 12 months of age, animals in the EX group began a treadmill training program five days per week for 6 months. Behavioral data (memory, coordination, and strength) were collected at months 3, 6, 9, then monthly from 12-18. **RESULTS**: Females had greater grip strength relative to body mass compared to males and exercise attenuated the age-related and AD-induced decline. Females (WT & AD) performed better on the coordination task compared to males. Female AD-impaired memory was rescued with exercise, while males had no exercise-induced improvements. **CONCLUSIONS**: Exercise neuroprotection is a valuable therapy in AD and further research is needed to understand the mechanisms of exercise neuroprotection, specifically in the sex differences documented in this experiment.

84. Title: Does Partner Familiarity or Partner Experience Affect Avoidance Learning in Rats?

Authors: Ivy Auletti, Shannon Ruble, Cassandra Kramer, Lexe West, Allison Drouhard, Emma Wrampe, Charlotte Kettler, Jyothi Kalarikkal, Maria M. Diehl

Affiliation:
Department of Psychological Sciences, Kansas State University, Manhattan, Kansas, USA

Background and Objective:
To understand how partner type influences avoidance, we used Platform-mediated avoidance (PMA), a task in which rats forego rewarded lever pressing to avoid a footshock by stepping onto a platform (Bravo-Rivera, et al., 2014). We modified the task to enable rats to learn together. Previous research demonstrated that the presence of a cagemate enhances fear learning (Jeon et al., 2010). Our study builds on this work by investigating whether familiarity or experience affects avoidance.

Methods:
During social partner PMA, rat pairs were either naïve to the task, or one rat had prior training (avoidance demonstrator) (n=20). Rats trained as demonstrators were paired with their familiar cagemate, whereas naïve-naïve pairs were noncagemates and unfamiliar with each other. Animals were separated by a perforated plexiglass barrier with access to their own platform and lever and conditioned for 10 days (9 tone-shock pairings per day). Avoidance, freezing, lever-pressing, and time in the interaction zone, a location adjacent to the platform (and barrier). These measures were compared between familiar pairings (demonstrators) and unfamiliar pairings (Naïve-Naive pairs), as well as partner type (Naïve-Naïve vs Naïve-Avoid). Avoidance was calculated as time spent on the platform during the tone.

Results:
Naïve- Naïve rats showed no significant difference in avoidance, time in the interaction zone, or pressing compared to Naïve- Avoid rats. Naïve-Naive rats showed slightly greater freezing beginning of each training day, but this was not significant. Naïve- Naïve and Demonstrators exhibited avoidance at a similar rate. Sex differences across partner type and familiarity were not observed. Taking into account familiarity and partner type, females spent more time in the interaction zone compared to males, but it was not significant.

Discussion and Conclusions:
Our current findings suggest that avoidance occurs at a similar rate regardless of partner type or familiarity. Future experiments will determine how dominance status or synchronized behaviors influence between partners avoidance.

Bravo-Rivera, Christian & Roman-Ortiz, Ciorana & Brignoni Perez, Edith & Sotres-Bayon, Francisco & Quirk, Gregory. (2014). Bravo-Rivera et al 2014.

Jeon, D., Kim, S., & Chetana, M. (2010). *Observational fear learning involves affective pain system and cav1.2 ca2+ channels in ACC*. Nature neuroscience. Retrieved April 11, 2023, from https://pubmed.ncbi.nlm.nih.gov/20190743/

Acknowledgements:
This work was supported by the Cognitive and Neurobiological Approached to Plasticity (CNAP) from the NIGMS (#P20-GM113109), the Kansas iDeA Network of Biological Research Excellence (K-INBRE) from the NIGMS (#P20-GM103418), and the Department of Psychological Sciences.

85. Determining the effect of the *sas-6(L69T)* primary microcephaly-associated mutation on ciliogenesis in *Caenorhabditis elegans*

Dierlam, Carter H[1], Smith, Ellie[1], Duran, Ramon[1], Iyer, Jyoti[1]

1. Department of Chemistry and Biochemistry, College of Engineering and Natural Sciences, The University of Tulsa, Tulsa, Oklahoma

Abstract

1. **Background and Objective:**
   Cilia are hair-like projections involved in cell motility and cell signaling and are generated through a process called ciliogenesis. Ciliogenesis defects have been linked with a neurodevelopmental disorder called primary microcephaly (MCPH). A previous study identified a mutation in the *SASS6/sas-6* gene to be associated with MCPH in humans. *C. elegans* were the first organism that *sas-6* was identified in, and the protein is functionally conserved between humans and *C. elegans*. Therefore, we used *C. elegans* as a model to investigate if the MCPH-associated *sas-6(L69T)* mutation has any effect on ciliogenesis. In *C. elegans*, the only ciliated cells are the sensory neurons. Our objective was to introduce the MCPH-associated *sas-6(L69T)* mutation into *C. elegans* and observe what, if any, effects the mutation has on ciliogenesis.
2. **Methods:**
   To generate the *sas-6(L69T)* mutation, CRISPR/Cas9 was utilized to edit the *C. elegans* genome. Once the edit was confirmed, the mutation was introduced by genetic crossing into a pan-ciliary marker strain. The generation of this strain enabled us to visualize if introducing the MCPH-associated *sas-6(L69T)* mutation caused any cilia defects in the *C. elegans.*
3. **Results:**
   Our studies revealed that phasmid cilia that are present in the tail region of worms were significantly shorter in *sas-6(L69T)* mutant worms. Specifically, the average phasmid cilia length in control worms was 5.8 μm (n=26) while that in the *sas-6(L69T)* worms was 4.71 μm (n=35).
4. **Discussion and Conclusions:**
   Based upon our results, we conclude that the *sas-6(L69T)* mutation causes ciliogenesis defects such as reduced ciliary length in worms carrying this mutation. As ciliogenesis defects have been linked with the incidence of MCPH, we propose that the ciliogenesis defects that are caused by the *sas-6(L69T)* mutation could likely contribute to the incidence of MCPH in individuals carrying this mutation.

Acknowledgements:

Centers of Biomedical Research Excellence (COBRE) grant 5P20GM103636-09

University of Tulsa Undergraduate Research Grant Shark tank funding

Faculty Summer development grant

CSURP Program

TURC Program

The University of Tulsa's Department of Chemistry and Biochemistry

Piali Sengupta Lab, Brandeis University

86. Cerebellar Volumes in Valproic Acid-Exposed Rats Model Human ASD Pathology Throughout Development

C. King[1], I. Mali[2], M. Payne[2], T.R. Maze[1], A. Pahua[1], S.H. Bossmann[2], B. Plakke[1]

[1]Department of Psychological Sciences, Kansas State University, [2]Department of Chemistry, Kansas State University, Manhattan, KS 66506

Background/Objective: One phenotype of Autism Spectrum Disorders (ASD) is dysregulation of brain volume throughout development. Studies with humans and animal models have found overgrowth in cortical regions and undergrowth in cerebellar regions and different developmental ages. Cerebellar changes have been linked to ASD symptomology. Here, maternal exposure to valproic acid (VPA) was used to induce ASD-like phenotypes in rats and cerebellar volumes were measured in early and late adolescence.

Method: Pregnant Long-Evans dams were injected intraperitoneally with 600 mg/kg sodium valproate (VPA) or vehicle at gestational day 12.5. One female and one male pup from each litter was assigned to Postnatal day 28 or Postnatal day 40 cohort. Brains were scanned with magnetic resonance imaging at P28 or P40. Volumetric measurements were segmented with ITK snap by blind to condition trained personnel. Volumetric data were normalized to total brain volume and analyzed by region and age.

Results: VPA-treated animals had decreased total cerebellar volume in both early (P28) and late (P40) adolescence, with trends approaching significance for the P28 cohort ($p = 0.069$) and reaching significance for the P40 cohort ($p = 0.0056$). Crus I, a subregion of the cerebellum, was not different between VPA and control rats, which suggests the decreased volumes found in adult animals in our prior study occurs due to faster pruning after adolescence.

Discussion: These findings parallel findings in the human literature of volumetric dysregulation in the cerebellum throughout development and show that the VPA model captures similar pathology in this regard to ASD. These data also align with the lab's previous work examining region-specific volumetric changes in adolescence and adulthood. These results provide insights into the dynamic nature of brain volume regulation across development within a model of ASD. Understanding these trajectories may lead to better interventions to target specific brain areas at different ages.

Grant Support: This research was supported by funds from the NIH under grant numbers P20GM103418 and GM113109

87. Investigating carbamoylated erythropoietin as a pharmacological treatment for autism spectrum disorder

Alexander D. Kloth[1], Sean W. Lemke[2], Amaya L. Street[3], Kevin M. Schumacher[1], Liza M. Schoenbeck[1], Vedant Thakkar[1], Samuel Sathyanesan Newton[4]

Departments of Biology, Psychology[2], and Chemistry and Biochemistry[3], Augustana University, Sioux Falls, SD; Center from Brain and Behavior Research, University of South Dakota, Vermillion, SD[4]

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects over 2% of the population worldwide, which is characterized by repetitive behaviors, restricted areas of interest, deficits in social communication, and high anxiety. Currently, there are no known effective treatments for the core features of ASD. Previous literature has established erythropoietin (EPO) as a promising antidepressant, working as a neurogenic and neurotrophic agent. However, EPO is also associated with an increase in red blood cell production, leading to high blood pressure, fever, and edema amongst other negative conditions. Carbamoylated erythropoietin (CEPO), a newly synthesized compound from the Sathyanesan group at the University of South Dakota, appears to retain the neuroprotective factors of EPO without the hematologic properties. Using an idiopathic ASD mouse model (BALB/c), we investigated whether CEPO can recover deficits in social behavior and anxiety which are associated with ASD. BALB/c mice score markedly higher in scales of anxiety and lower in sociability. C57 mice were used as controls. After an injection period of CEPO (40 µg/kg in PBS) or vehicle over 21 days, we analyzed the behavior in the three-chamber social approach, the open field, the elevated plus maze, and the Porsolt's forced swim tests. Preliminary evidence suggests a rescue of heightened anxiety and lower sociability, suggesting that CEPO may be an effective pharmacological option for treating ASD. Upon completion of behavior testing, brain tissue samples will be harvested to examine both the gross physical structure and levels of gene expression in brain structures related to behaviors of anxiety and sociability.

88. Title:

Photosilencing Anterior Cingulate Cortex Neurons Impairs Active Avoidance under social conditions in rats.

Authors:
Cassandra Kramer, Shannon Ruble, Lexe West, Ivy Auletti, and Maria M. Diehl.

Affiliation:
Department of Psychological Sciences, Kansas State University, Manhattan, Kansas, USA.

Text:

Background and Objective:
Previous studies have utilized the platform-mediated active avoidance (PMA) task, in which rats avoid a tone-signaled shock by stepping on a platform at the cost of receiving a sucrose reward under solitary conditions in male rats (Diehl, et al., 2019). The current study aims to understand how male and female rats acquire PMA with a social partner separated by a perforated plexiglass barrier, allowing rats access to their own lever and platform. We were further interested in whether activity within the anterior cingulate cortex (ACC) was necessary for PMA under social conditions, given its known role in social learning (Apps, et al., 2016).

Methods:
We applied an optogenetic approach to photosilence ACC activity during expression of PMA. Following surgery, male and female rats (n=46) were trained in PMA under solitary or social conditions. Following 10 days of training, ACC was photosilenced during Tone 1 of a 2-tone test of avoidance expression (social PMA rats underwent 2 tests, in the presence or absence of their partner). Avoidance was measured as time spent on the platform during the tone and compared across conditions.

Results:
Rats trained under social conditions showed similar levels of avoidance, but greater freezing ($p<0.001$) compared to rats trained under solitary conditions, suggesting that the partner acts as a contextual cue. Females showed increased avoidance compared to males ($p<0.001$) under solitary conditions, but not under social conditions. Photosilencing ACC impaired avoidance when the partner was absent ($p=0.015$), but had no effect when the partner was present nor any effect on solitary rats.

Discussion and Conclusions:
These results suggest that ACC may be recruited for recalling the social memory of avoidance when the partner is absent. This research will further our understanding of neural circuits disrupted in neuropsychiatric diseases involving maladaptive avoidance and social factors that influence avoidance behaviors.

Acknowledgements:
This work was supported by the Cognitive and Neurobiological Approached to Plasticity (CNAP) from the NIGMS (#P20-GM113109), the Kansas iDeA Network of Biological Research Excellence (K-INBRE) from the NIGMS (#P20-GM103418), and the Department of Psychological Sciences.

89. Effects of Whole-Body Resistance Exercise in Young and Middle-Aged Rats

Ava K. Lee[1], Fu-Chen Yang[1], Paige Morefield[1], Paul Kueck[2], Olivia J. Veatch[3], Jill K. Morris[2], John A. Stanford[1]

[1]Department of Cell Biology and Physiology; [2]Department of Neurology, [3]Department of Psychiatry and Behavioral Sciences; University of Kansas Medical Center, Kansas City, Kansas, 66160, United States

Preclinical studies can reveal mechanisms underlying the effects of exercise. However, most animal studies have focused on aerobic exercise. Our goal was to compare effects of a whole-body, progressive overload resistance exercise protocol in young adult and middle-aged rats. We trained ten male rats (3-5 month-old) to climb a ladder with weights attached to their tails. The protocol then determined maximum weight loads (Mondays) and repetitions with increasing percentages of maximum weight (Wednesdays and Fridays) for eight weeks. One year later we repeated the protocol with four of the previously trained rats, leaving five as sedentary controls. Although the maximum load carried was similar for the rats when they were young vs older, the ratio of maximum load to body weight was greater when they were young (1.2-1.5) vs older (0.88-1.4). The rats reached the goal box quicker when they were older (~6s) vs younger (~8s). Lean mass increased with resistance exercise more when the rats were young (28%) than older (10%). Fat mass increased slightly in the young rats (12%) but decreased with exercise when they were older (39%). Blood levels of neurofilament light (NFL; neurodegeneration maker) and glial fibrillary acidic protein (GFAP; inflammation marker) increased 8-fold and 3-fold, respectively, from young adult to middle-age. Resistance exercise had no effect on NFL and GFAP in the older rats. Our results reveal age-related differences in the effects of resistance exercise on body composition in rats. Preliminary proteomic data from hippocampus and striatum tissue samples in the older exercised vs sedentary rats will also be presented.

90. Title: Exercise modulates cognitive performance in the rat model of Autism

Authors: Alma E. Pahua, Cole King, Ivina Mali, Macy Payne, Stefan H Bossmann
Background and Objective: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by impaired social communication, restricted patterns of behavior, sensitivity to change, and high anxiety levels. Previous research has implied a critical role of brain changes in the developmental trajectory and executive function of ASD individuals. Using the Valproic Acid (VPA) model of ASD, the current experiment analyzed structural differences in the amygdala and hippocampus after an exercise intervention and performance on set-shifting.
Methods: Pregnant Long-Evans dams were injected with either saline or VPA (600mg/kg valproic acid) on day 12 of gestation. One male and a female pup were pulled from every litter and assigned to different experimental conditions to control for the litter effect. Using a rodent treadmill, animals began exercising on Postnatal day 40 and ran on a 0% slope for 30 minutes/day, 5 days/week for 4 weeks. During the fourth week of running, attentional set-shifting was conducted, followed by 3D MRI scans.
Results: Regardless of exercise, VPA rats demonstrated an enlarged amygdala compared to the control animals. For the hippocampus, it was found that control animals who received the exercise intervention had larger volumetric measures compared to the sedentary control animals. In addition, error probabilities (preservative and regressive) were also analyzed. It was found that female VPA rats had more preservative errors compared to the female control rats. For regressive errors, sedentary rats displayed a greater number than exercise rats.
Discussion and Conclusions: These results align with findings in the clinical populations, suggesting that exercise mitigates error rates in the VPA animals, but is not reliant on changes in brain volume.

Funding awarded by P20GM103418

91. Analysis of OSCP Deregulation in Alzheimer's Disease

Albert Park[1], Tienju Wang[1], Jing Tian[1], Kun Jia[1], Lan Guo[2] Heng Du[1,2]

Pharmacology and Toxicology Department, University of Kansas, Lawrence KS, USA[1]

Higuchi Biosciences Center, University of Kansas, Lawrence KS, USA[2]

Alzheimer’s disease (AD) is a chronic and irreversible neurodegenerative disease that affects more than 44 million people worldwide. There are currently no effective therapies for this devastating neurological disorder and the detailed molecular mechanisms of AD etiopathogenesis remain unelucidated. Mitochondrial dysfunction is emerging as a vital contributor to the development of AD. The molecular pathways that lead to disease-associated mitochondrial abnormalities including impaired ATP production via oxidative phosphorylation (OXPHOS) are under intensive investigation. Previous studies have implicated loss of oligomycin sensitivity conferring protein (OSCP), a key protein of the F1Fo ATP synthase, in AD pathology; however, the precise mechanism for OSCP degradation in AD conditions remains unknown. Our study aims to establish an age-dependent deregulation of ubiquitinated-OSCP in a mouse model of AD. We have discovered increased ubiquitinated OSCP in a mouse model of AD as compared to their wildtype age-matched counterparts. In contrast, the treatment of amyloid-beta (Aβ) onto primary cultured cortical neurons revealed increased expression of OSCP. This may be a result of cellular compensation for the increased ubiquitinated OSCP seen in vivo. Further studies will be performed to add clarity to OSCP degradation in an AD context and will be a key to understanding the precise mechanism behind OSCP loss in AD and integrating it as a potential therapeutic target in AD.

This study is supported by research funding from NIH (National Center for Research Resources (P20RR016475) and the National Institute of General Medical Sciences (P20GM103418) from the National Institutes of Health.

92. Title: Effect of Treadmill Training in a Rodent Model of Autism Spectrum Disorder

Authors: Liza Rogers, Stephanie E. Hall, Alma Pahua, Cole King, Kiana Schulze and Bethany Plakke

Institutions: Kansas State University

Background: Autism prevalence has increased 175% since 2000 with 1% of the world's population identified with autism spectrum disorder (ASD). Today, ASD affects 1 in 44 children and is characterized by delayed language, motor and cognitive skills. **Purpose:** this project will provide a better understanding into the pathology and the potential impact of exercise. **Methods**: Sixteen Long-Evans pregnant rat dams were injected with a single dose of either saline (n=5) or VPA (Sodium Valproate (sigma), 250mg/ml, mixed in saline, 600 mg/kg, n=11). This prenatal exposure to VPA is known to increase the risk of ASD development in offspring. Rats were provided with free access to food, pair-housed with a light cycle of 7am-7pm. Rats began a 4-week exercise protocol on the treadmill (Harvard Apparatus, Holliston, MA) on post natal day 40. Rats ran 5 days a week for 40 minutes at 5-13 cm/s at 0^{o} inclination. Motor coordination was measured with a rotarod both pre and post intervention. The rod maintained a constant 5rpm speed and the trial ended after 180 seconds. Latency time was recorded for each trial. Following behavioral testing, brain and skeletal muscle tissues were collected. Skeletal muscle (soleus) was homogenized and western blots were conducted to quantify protein expression. **Results**: While not statistically significant due to high variability, there was a trend toward reduced motor coordination in the VPA groups which was improved with exercise. In order to confirm an exercise effect, citrate synthase expression was quantified in the soleus muscles. Treadmill trained animals trended towards higher CS expression compared to sedentary animals (not statistically significant). **Conclusion**: The protective effect of exercise against ASD pathology is important to understand both in its use as a therapy but also as a tool to highlight the protective pathways.

93. How does Observational Social Learning Differ from Simultaneous Social Learning of Active Avoidance in Rats?

Authors: Shannon Ruble, Cassandra Kramer, Lexe West, Ivy Auletti, Allison Drouhard, Maria M. Diehl

Affiliation: Department of Psychological Sciences, Kansas State University, Manhattan, Kansas, USA

Background and Objective:

Learning to avoid danger can be achieved through direct or indirect experience. Observational learning, achieved through indirect experience, has previously been demonstrated in shuttle avoidance (Del Russo, 1975) but has yet to be studied in platform-mediated avoidance (PMA). In PMA, rats learn to avoid a tone-signaled shock by stepping onto a platform at the cost of access to a sucrose reward (Bravo-Rivera, et al. ,2014). Here, we developed a novel observational PMA task and assessed how rats acquired PMA directly when learning simultaneously with a partner versus indirectly through observation.

Methods:

During social partner PMA, pairs of rats were simultaneously conditioned while separated by a perforated plexiglass barrier for 10 days and received 9 tone-shock pairings per day. During observational PMA, Observers were randomly assigned to watch a Demonstrator after one of three timepoints during conditioning: day 1 (D1), days 2 through 9 (D2-9), or day 10 (D10). After training, Observers were placed on the same side of the apparatus that the Demonstrator previously occupied and underwent a PMA test. Avoidance and freezing were compared in rats that learned social partner or observational PMA.

Results:

Rats who learned social partner PMA (n=22) showed no difference in freezing or avoidance on day 1 of conditioning compared to D1 Observers (n=4). D1 Observers (n=6) showed no difference in freezing but significantly lower avoidance (t(8)=-2.74, p=0.026) compared to D10 Observers. There was no significant difference in freezing or avoidance between rats who learned social partner PMA across 8 days and D2-9 Observers.

Discussion and Conclusions:

Our findings suggest that avoidance learned indirectly (via observation) is similar to avoidance that is learned directly. Our findings also suggest that Observers show enhanced learning with an experienced Demonstrator compared to a naïve Demonstrator. Further research will characterize how previous experience with shock and lever-pressing influence observational learning of avoidance.

Grant Support: This work was supported by the Cognitive and Neurobiological Approached to Plasticity (CNAP) from the NIGMS (#P20-GM113109), the Kansas iDeA Network of Biological Research Excellence (K-INBRE) from the NIGMS (#P20-GM103418), and the Department of Psychological Sciences

94. Abstract

Effects of intermittent fasting on brain and skeletal muscle tissue in male Fisher-344 rats.

Keshari Sudasinghe, Zachary White, Liza Rogers, Stephanie Hall

Background and Objectives: Intermittent fasting (IF), time-restricted eating, has been shown to improve cognition in animal models through the regulation of protein synthesis (mTOR pathway) and oxidative stress damage (antioxidant expression). We hypothesized that IF would improve antioxidant expression and reduce protein synthesis (via mTOR expression). **Methods**: Twenty-four, 10-week-old male Fisher-344 rats were randomly assigned into two groups (ad-libitum, or IF) and housed 4 per cage, on a 12:12 light: dark cycle. Ad-libitum group had 24/7 access to food and water while the IF group had 24/7 access to water and full access to food on an every-other-day basis for ten weeks. At 20 weeks of age, animals were euthanized and brain (hippocampus and cortex) and skeletal muscle (soleus and extensor digitorum longus) tissues were sectioned, and snap-frozen. Following homogenization, Jess Protein Simple automated western blotting (Bio-Techne, Minneapolis, MN) was used to quantify mTOR (Santa Cruz, sc-517464), and SOD2 (Abcam, ab68155). Compass software-generated *area under the curve* values were used to determine protein expression and analyzed with a two-tailed T-test per Prism Graph Pad software. **Results:** In cortex tissue and skeletal muscles, mTOR expression was significantly reduced in the IF group compared to the AL group ($p<.001$, $p<0.05$), however, mTOR expression was not significantly different in the hippocampus. Antioxidant enzyme, SOD2, was significantly higher in IF cortex tissues ($p<.05$) compared to AL controls. In contrast, SOD2 was significantly lower in IF hippocampal tissues ($p<.01$) compared to controls. SOD2 expression was not significantly different in the skeletal muscles. **Conclusion**: In the present study, IF significantly impaired protein synthesis (via mTOR expression) in the brain cortex and skeletal muscles. However, the fact that no decline was present in the hippocampus and the reduction of the antioxidants in the hippocampus were intriguing and will be the focus of follow-up investigations.

Funding Source: CNAP / P20GM113109

Scientific Focus Areas: Neuroscience/ Nutrition and Diet

Title: Effects of intermittent fasting on brain and skeletal muscle tissue in male Fisher-344 rats.

Author(s): Keshari Sudasinghe (BS, MS), Zachary White (BS, MS), Liza Rogers (BS), Stephanie Hall (BS, MS, Ph.D.)

Affiliation for all authors: Anatomy and Physiology Department, College of Veterinary Medicine Kansas State University Manhattan

Acknowledgments: Grant support: CNAP (Cognitive and Neurobiological Approaches to Plasticity) **(**Funding Source: P20GM113109)

95. Title: How do traumatic experiences alter social interactions and the associated neural mechanisms?

Authors: Lexe West, Viviana Valentin-Valentin, Shannon Ruble, Cassandra Kramer, Greg Erickson, Maria M. Diehl

Affiliation:

Department of Psychological Sciences, Kansas State University, Manhattan, Kansas, USA.

Department of Psychiatry, University of Puerto Rico School of Medicine, San Juan, PR, USA.

Background and Objective:

Exposure to traumatic experiences can heighten stress and anxiety and subsequently alter social behaviors, but the presence of a social partner can buffer fear responses (Kiyokawa et al., 2007). To study this in the lab, rats underwent platform-mediated active avoidance (PMA), in which they learned to avoid a tone-signaled shock by stepping onto a platform at the cost of reward seeking in the presence or absence of a partner. We then assessed a rat's willingness to investigate a conspecific using the social exploration task. To elucidate the neural mechanisms of social interactions following PMA training, we optogenetically activated or inhibited brain regions recruited during avoidance and social behaviors.

Methods:

During the social exploration task, a PMA-trained rat can investigate an unfamiliar conspecific confined in a cage in the center of an open field. Time spent near the center cage and overall freezing were analyzed in the presence and absence of the unfamiliar conspecific to assess levels of social exploration. We optogenetically photo-stimulated projections from the prelimbic (PL) cortex to the nucleus accumbens (NAc), a pathway implicated in mediating social interactions (Murugan et al., 2017). We also photo-inhibited projections from the PL to basolateral amygdala (BLA) and anterior cingulate cortex (ACC) neurons, regions implicated in social learning (Burgos-Robles, 2019; Felix-Ortiz et al., 2016).

Results:

Photo-stimulating PL-NAc projections and PL-BLA projections had no effect on social exploration, locomotion, or freezing. Photo-inhibiting ACC neurons also had no effect on social exploration behaviors. However, when ACC neurons were photo-inhibited, freezing significantly increased in rats trained in PMA under solitary conditions compared to social conditions.

Discussion and Conclusions:

Having a social partner p

resent during a traumatic experience may buffer fear responses in subsequent social situations. Future studies should examine how these experiences alter other social behaviors such as social dominance and observational learning.

Acknowledgements:

This work was supported by the Cognitive and Neurobiological Approached to Plasticity (CNAP) from the NIGMS (#P20-GM113109), the Kansas iDeA Network of Biological Research Excellence (K-INBRE) from the NIGMS (#P20-GM103418), and the Department of Psychological Sciences.

96. Influence of Aerobic Exercise on Cognitive Function in the Tgf344-AD Model of Alzheimer's Disease

Authors: Zachary White[1], Michael Young[2], Stephanie Hall[1]

Affiliations: [1]Department of Anatomy and Physiology, Kansas State University; [2]Department of Psychological Sciences, Kansas State University

Background and Objective – Exercise is a promising strategy for Alzheimer's disease (AD) protection although our understanding of exercise-induced neuroprotection remains incomplete. The goal of the present study is to longitudinally characterize the influence of exercise training on cognitive function in male and female TgF344-AD rats. We hypothesize that cognitive function will be impaired by AD and exercise-training will rescue AD-induced impairments.
Methods – Twelve-month-old male and female TgF344-AD rats (n=62) underwent a six-month progressive treadmill training protocol. Training was conducted 5 days/week and intensity maximized at 24 meters/minute for 60 minutes. Testing was conducted longitudinally pre- and post-intervention to assess spatial learning and memory, motor coordination, and muscular strength. Investigators were blind to AD presence. Differences were identified with Bayesian multivariate linear regression.
Results – Memory (latency time, s) was rescued by exercise in AD females AD. Post-intervention, female exercise groups demonstrated improved memory (-0.02 log scale s/m) while sedentary controls declined; no exercise effect was detected in male wild-type (WT) or AD groups. Post-intervention, average grip strength (grams force/grams body mass) declined across all groups and declines were greater in AD compared to WT animals (-0.038 vs -0.011 log-transformed g/g per month). Exercise attenuated the age-related declines compared to sedentary controls (0.001 vs -0.0498).The rate of grip strength loss from 13 m to 18 m was one-eighth the rate in female AD exercisers compared to female AD sedentary (-0.009 vs -0.075) while male AD exercisers lost strength at one-fourth the rate compared to male AD sedentary (-0.013 vs -0.054).
Discussion and Conclusions – Influences of advanced age, AD genotype, and exercise training in the TgF344-AD rat manifested in a sex-specific manner. Exercise training ameliorated AD-and age-induced memory and strength deficits in females to a greater degree than males. Our time-wise characterization of the TgF344-AD rat provides reference for future biochemical analysis aimed at elucidating mechanisms of exercise-induced neuroprotection.

Acknowledgements: R03AG065970, P20GM113109

97. The Feasibility of Utilizing the Open Dynamic Interaction Network (ODIN) app to assess rEMA data across 30 days among those Recovering from Alcoholism

Jessica Phelps, Rural Drug Addiction Research Center, University of Nebraska-Lincoln; Dennis McChargue, Department of Psychology, University of Nebraska-Lincoln; Bilal Khan, Department of Community and Population Health, Lehigh University; Patrick Duryea, Department of Psychology, University of Nebraska-Lincoln; Trey Andrews, Department of Psychology, University of Nebraska-Lincoln; and Kim Tyler, Department of Sociology, University of Nebraska-Lincoln

Abstract

Background and Objective: Over 28 million people have been classified as having an alcohol use disorder over the past year. Those who receive treatment (~4 million/year) frequently relapse producing a seemingly rapid revolving door between recovery and use. This paper presents preliminary data from a prospective micro-longitudinal study (30 days) that examines co-evolution of relapse risk processes of people with alcohol use disorders who entered a short-term residential substance use treatment. The primary goal of the current data was to assess the feasibility of using the ODIN responsive ecological momentary assessment (rEMA). **Methods**: rEMA collected daily estimates on affect, urges, sober-support engagement, and use. Twelve questions were asked at random times each day. Additional questions were prompted from GPS identified engagement in sober support activities and alcohol cue exposure (e.g., gas stations, grocery stores, bars). **Results**: Of the 800 questions, the mode for questions answered was 500. Five-day estimates showed that 80% of the participants answered between 80-100 questions (10-30 questions/day). Also, 95% of GPS readings were acquired across 30 days (~288 GPS readings/day). Most were satisfied with stability (89%), look/feel (77%), quality (71%) and ease of use (80.4%) of the ODIN app. Participants also reported interest in longer assessments (81%), recommending the study (71%) and using the app if it prompted them to call sponsor (82%) or to use relapse prevention skills (77%).

Discussion and Conclusions: Preliminary findings show that data can accurately and efficiently be collected amongst this population. There was a high rate of acceptability, satisfaction, and interest in using ODIN to help them engage in sober support/relapse prevention.

Acknowledgments: This research is funded by a grant from the National Institute on alcohol Abuse and Alcoholism (NIAAA) 1R21AA029231. Dennis McChargue & Bilal Khan, MPIs.

98. Title: Research for the Common Good: Can we trust lead-free marketing for tableware?

Authors: Qiyang Zhang

Physical Sciences, Emporia State University

Abstract: Lead is frequently used in paint and glazes that seal earthen tableware. Since the 1960s, child lead poisoning has become a public issue (Rabin, 1989). Identifying lead poisoning can be challenging, because the symptoms frequently seem very benign and manifest in different ways such as gastrointestinal problems, delays in physical development, and impaired neurologic development (Warniment et al., 2010). Even exposure to very low levels of lead is associated with significant loss if IQ during childhood; hence, to protect public health, near-zero levels of exposure to toxins are essential (Lanphear, 2017).

Like lead, cadmium also interferes with the central nervous system and other body functions (Flora, 2009). It is used as a pigment and produces vibrant yellow, orange, and red colors and glazes (OSHA). This toxin has a long half-life and kidneys retain cadmium for 10-30 years (Clemens et al., 2013).

To the best of our knowledge, claims of lead-free and cadmium-free tableware has not been tested and evaluated. We will use two main methodologies to measure the levels of lead and cadmium in drinkware: method by Food and Drug Administration (FDA Elemental Analysis Manual, Jan. 2020), and the U.S. Environmental Protection Agency's (EPA) procedure (EPA method 3050b). We will either confirm that consumers can trust such claims, that the claims are false, or that only certain brands fulfill their marketing claims.

99.

The Relevance of Knowledge of Network Science and Awareness of Social Connections for Health for Pandemic Precautions

Julia McQuillan[1], Patricia Wonch Hill, Joseph Jochman, Liz VanWormer, Amy Spiegel, Meghan Leadabrand, Judy Diamond
[1]University of Nebraska

Is awareness of social connections - implicit social network knowledge, and explicit knowledge of social network research related to health associated with taking pandemic precautions? Social network science measures the structure of relationships among people and is often used in public health. Increased media coverage of network science (e.g. contact tracing) during the COVID-19 pandemic could have helped more people define the pandemic as a situation in which precautions made sense, even if political rhetoric and misinformation created confusion. Network science is applicable to many health outcomes, and was developed particularly for research on substance use research. In this study we test the associations of implicit awareness of social connections and explicit knowledge of network science applied to the outcome of pandemic precautions using survey data collected in the summer of 2020, before there was a vaccine. We used survey data from a panel of Nebraskans (N = 475). Results indicate that network science awareness and knowledge are associated with reporting taking more pandemic precautions, adjusted for relevant social statuses and general knowledge of COVID-19.

The content is supported by the Worlds of Connections SEPA (Science Education Partnership Award) [R25GM129836] at the University of Nebraska - Lincoln, funded by the National Institute of General Medical Science of the National Institutes of Health. The content herein is solely the responsibility of the creators and does not necessarily represent the official views of the National Institutes of Health of the University of Nebraska.

100. Open Dynamic Interaction Network (ODIN) app with Youth Experiencing Homelessness: Preliminary Results and Feasibility over 60 Days

Kimberly A. Tyler

Willa Cather Professor of Sociology

University of Nebraska-Lincoln

Co-Director, Longitudinal Networks Core

Rural Drug Addiction Research Center

Bilal Khan

Professor of Data Science and Director of the Health Data Warehouse

Lehigh University College of Health and Department of Computer Science

Kristen Olson

Leland J. and Dorothy H. Olson Professor in Sociology
University of Nebraska-Lincoln
Director, Bureau of Sociological Research

Abstract

Background and Objective: U.S. estimates reveal 700,000 youth and 3.5 million young adults experience some form of homelessness each year making homelessness a major public health issue. These young people face many significant risks that are detrimental to their long-term well-being and survival, including engaging in alcohol and drug use. Drug and alcohol use prevalence rates are 2 to 3 times higher among youth experiencing homelessness (YEH) than in their housed peers, leading to poorer mental health and HIV outcomes for this vulnerable population. This paper presents preliminary data from an ongoing longitudinal study of YEH in the Midwest to assess feasibility of using the Open Dynamic Interaction Network (ODIN) app with this population. **Methods**: We use surveys at baseline, 30-days, and 60-days to collect data on family histories, street experiences, service usage, and substance use. We also employ ecological momentary assessment (EMA) to collect fine grained daily data from participants. Using an Android phone with the ODIN app, participants were asked up to 18 questions per day about service utilization, support, mental health, and substance use. **Results**: Results show that youth who enrolled in the study in the month of November, for example, answered on average between 70 and 90% of daily questions. For those who enrolled in January, the percentage of daily questions answered by youth, on average, was between 62 and 84%. In terms of the distribution of participation levels, the number of questions answered by youth follows a normal distribution, with most respondents answering 56% or more of the daily questions. **Discussion and Conclusions**: Initial findings suggest that many youth will answer daily EMA questions with regularity. Findings also have implications for those who work with YEH and who want more fine-grained data to assess current behaviors particularly using substances to cope with daily street life.

Acknowledgments: This research is funded by a grant from the National Institute on Drug Abuse (NIDA) 1R01DA054969-01A1. Kimberly A. Tyler, PI. Support for this conference presentation is provided *by the National Institute of General Medical Sciences of the National Institutes of Health [P20GM130461]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.*